HERITABLE VARIATIONS IN *NICOTIANA TABACUM* L. INDUCED BY ABNORMAL TEMPERATURES, AND THEIR EVOLUTIONARY SIGNIFICANCE[1]

By D. KOSTOFF and M. SARANA

(With Plates XVIII and XIX and Six Text-figures)

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Introduction

After the appearance of Darwin's (1859, 1868) classical works upon the origin of species, further studies upon the problem of evolution were developed chiefly into two main directions: (1) searching for transitional forms for the completion of phylogenetic trees in the plant and animal kingdoms, (2) investigations (chiefly experimental) upon the nature and the causes of the heritable variations. These investigations have been very fruitful, especially during the last 15 years, since various kinds of heritable variations have been experimentally induced by several environmental factors (X-ray and radium irradiations, abnormal temperatures, chemical agents, etc.). There is no doubt that abnormal temperatures are one of the most powerful factors in nature for inducing heritable variations. As demonstrated by many workers, high and low temperatures interfere with the procedure of the meiotic and mitotic

[1] This work was started in 1930 when the senior author was working at Sofia University. It was later continued chiefly at the Tobacco Institute in Krasnodar. Some variants and their hybrids were recently grown at the Academy of Sciences, Institute of Genetics, Moscow.

processes, influence the frequency of the chromosome interchanges in the somatic and generative cells, and, affecting the nucleus, condition various kinds of chromosome alterations and gene mutations.

In our experiments we applied both low and high temperatures. After the successful experiments of one of us (Kostoff, 1931*a*) by which plants with altered chromosome sets (numerically and structurally) were obtained in *Capsicum* under the influence of abnormal temperatures, further experiments of similar nature were designed in *Nicotiana tabacum*.

Material, methods and nomenclature

Nicotiana tabacum var. *macrophylla* was used for the experiments. This variety was inbred for several years at the Bussey Institution, Harvard University, before the beginning of the experiments and no noticeable variations have since been observed over twelve generations.

Seeds of twenty capsules were collected from a single plant after self-pollination in the greenhouse. The capsules, formed in the greenhouse, contained usually 900–1100 seeds (average content 1000), while those formed in the field contained about 1200–1500 seeds. Collected seeds were kept at 40–43° C. for 27 days. Two months later they were put for germination in small pots (4/10/12) with sterile soil at 38–40° C. for 3 days, at 20–27° C. for 4 days (greenhouse condition), and then transferred to a thermostat with glass walls at a temperature of 6–9° C. for 20 days. The seedlings were grown on under ordinary greenhouse conditions. When the largest reached a size of about 2·5–5 cm. all the normally developing ones were thrown away, while the smallest ones, and especially those of them that had somewhat deformed leaves, were transplanted. Forty-five plants of these types were selected and transplanted in pots. These plants were labelled 801. They will be called T_0 generation. Seeds collected from ten other capsules by self-pollination of *N. tabacum* var. *macrophylla* were used for control. They were sown when the treated seeds were sown, and forty of the smallest seedlings were selected and transplanted when the transplantation of the T_0 seedlings was carried out. Fourteen of the most morphologically outstanding T_0 plants (801) were each exposed three times for 22–23 hr. to a temperature of 38–41° C. in thermostats with glass walls (double) before the beginning of the formation of floral buds. Seven of them developed further in the greenhouse, while the other seven developed in large pots in the garden. When these fourteen T_0 plants formed floral buds they were exposed three times every second day to a temperature of 38–41° C. in glass thermostats for 6–6½ hr. Most of the floral buds of

seven T_0 plants grown in the greenhouse dropped when exposed to such a temperature, while the buds of the seven T_0 plants that grew in the garden stood the treatment better. The very few flowers that developed from the persisting floral buds had a large percentage of abortive pollen grains (15–70%), the viable ones being unequal in size. The plants of the controls grown in the greenhouse, as well as those grown in the garden, usually formed 96–99·5% viable pollen grains.

Each flower of the fourteen treated plants was carefully self-pollinated or pollinated with pollen grains from treated T_0 (801) sister plants having the greatest percentage of abortive pollen. Some of them dropped down without setting seeds, so that very few capsules were formed. Those of the plants that had capsules were again exposed 2–3 times for 6–7 hr. at a temperature of 39–41° C. Some of the capsules dropped down. Only six capsules with ripe seeds were collected. They had a small amount of normally developed seeds, amongst which shrunken seeds and black small ovules were found. We grew a T_1 generation from these seeds which consisted of about 200 plants, but we took about sixty (the smallest) of which thirty-six reached maturity, since a large number of the seedlings died at various stages of development. These thirty-six plants were studied. Six of them formed viable pollen grains (*ca.* 96–99·5%), while most of the others formed large percentages of abortive pollen grains (15–75%). High percentages of abortive pollen grains (35–75%) were displayed by the T_1 plants 801/1, 801/2, 801/5, 801/7, 801/8, 801/10, 801/12, 801/20, 801/21 and 801/24. These T_1 plants were used for further investigations. We applied numerous treatments to the T_0 plants (801), because we hoped to accumulate hereditary changes of various types. For the same purpose we crossed T_1 plant 801/1 with 801/2. Their T_2 progeny were further studied (801/1/20, 801/1/24, 801/1/25, 801/1/26). Plants obtained from the reciprocal cross 801/2×801/1 were further studied (T_2) under the following numbers: 801/2/1 to 801/2/21. From the cross 801/5×801/7 twenty-seven T_2 plants (801/5/1 to 801/5/27) were grown, while from the reciprocal cross twenty-three T_2 plants (801/7/1 to 801/7/23) were raised. From 801/8 with 801/7 twenty-three T_2 plants (801/8/1 to 801/8/23) were grown, while from the crosses 801/10×801/12 and 801/20×801/24 twenty-five and twenty-three T_2 plants were grown (801/10/1 to 801/10/25 and 801/20/1 to 801/20/23 respectively). No further crosses between the variants were carried out. The plants were subsequently propagated by self-pollination. Crosses were also carried out between the plants of the T_1 generation. Every care was taken to avoid cross-pollinations and accidental mixing of foreign

seeds. Seeds were sown in sterile soil or germinated in Petri dishes and then transplanted. Acetocarmine smear preparations and permanent paraffin and smear preparations were used for cytological investigations. Material for cytological studies were fixed in Bouin's fixative as modified by Allen, in a modification of Lewitzky's chromic acid (5 parts 2·5%) + formalin (5 parts 25%) fixative, in S. Navashin's fixative (10 parts 1% chromic acid, 4 parts 40% formalin and 1 part glacial acetic acid) and in La Cour 2 BE. Chromic acid-formalin fixative was used for fixing root tips, while Navashin's fixative and La Cour 2 BE were chiefly used for fixation of floral buds. The latter were killed first in Carnoy's fixative for 30–60 sec. and then transferred to Navashin's fixative. Allen's modification of Bouin's fixative was used for fixing both root tips and floral buds. Permanent preparations were stained by Heidenhain's haematoxylin and gentian violet.

Measurements of morphological characters were made at the florescence period in the field under practically equal environmental conditions. All leaves (upper, middle and lower) were collected for chemical analysis.

Drawings of the chromosomes were made with the Abbe drawing apparatus. Magnification *ca.* × 3000.

Biochemical analyses were carried out in the Biochemical Laboratory of the Tobacco Institute in Krasnodar by M. Khmura under the direction of A. A. Schmuck, for which we wish to express to them our gratitude. I. F. Zhiltzov painted the colour plate.

Meiosis in plants exposed to abnormal temperatures (T_0)

The plants exposed to low and high temperature showed abnormal meiosis, classifiable as follows:

(1) Reduction in chiasmata.

(2) Appearance of univalents.

(3) Laggards on the spindles during the first meiosis and occasionally during the second division.

(4) Formation of multivalents (rarely).

(5) Division of the lagging univalents during the first anaphase (rarely).

(6) Formation of chromatin bridges during the meiotic anaphase occasionally accompanied with small fragments (rarely).

(7) Appearance of fragments (rarely).

(8) Formation of restitution nuclei.

(9) Formation of micronuclei during the interkinesis on the spindle and during the second division.

(10) Appearance of second metaphases with unequal chromosome numbers due to non-conjunction and perhaps to non-disjunction. In a few cases the multivalents might be also responsible for this phenomenon.

(11) Formation of dyads, triads, unequal tetrads, pentads, etc.

(12) Formation of various numbers of abortive pollen and viable pollen, unequal in size.

Abnormal meiosis has been fully described in *Nicotiana* (Kostoff, 1930*a*, 1931*a*), in *Capsicum* (Kostoff, 1931*b*), in fruit trees (Heilborn, 1930; Kostoff, 1931*c*), and in wheat (Bleier, 1930; Sarana, 1930; and unpublished). Consequently therefore we have only briefly mentioned such characteristics of the meiosis in our treated plants of *N. tabacum* as suffice to explain the results obtained in selfing or crossing flowers with such meiosis.

MITOSIS IN PLANTS EXPOSED TO ABNORMAL TEMPERATURES (T_0)

The experiments of numerous investigators, and especially those of Kozhuchov (1928), Kostoff (1931*b*), Randolph (1932) and Peto (1935), showed that abnormal temperatures induce euploid and aneuploid chromosome alterations in the soma as well as exchanges of chromosome parts. It was of importance for the further development of our studies to find out whether the high temperatures applied in our experiments also induce such somatic alterations.

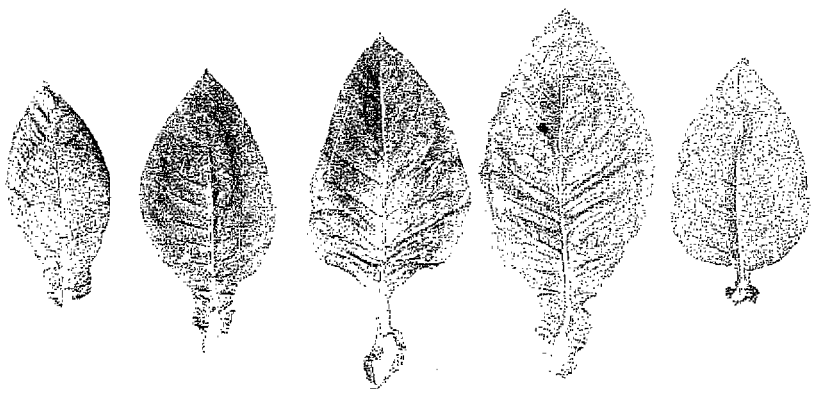
By exposing (2–4) small tobacco seedlings to a temperature of 38–42° C. several times for various periods (3–48 hr.) it was found that: (1) Polyploid sectors were formed in some root tips. (2) Chromatin bridges occasionally appeared during the anaphases (on longitudinal sections) which could be interpreted as a result of exchanges of chromosome parts leading to formation of bicentric chromatids. In a few instances chromatid exchanges in the root tips were found, like those described by Peto (1935) in *Hordeum*, and by Sax (1937) in *Tradescantia*. (3) Chromosome fragments were also found in a few instances. Such fragments were also reported by Sax (1937) in *Tradescantia*. (4) Aneuploid chromosome numbers were found in two instances.

Such irregularities in mitosis as well as in meiosis indicate that abnormal temperatures are a powerful factor in the induction of heritable variation.

Morphology and cytology of T_1 plants

The few seeds obtained from T_0 plants (801) exposed to abnormal temperatures were grown in sterile soil. Germination was poor and spread over a long period (4–21 days), while the control seeds germinated (*ca.* 98%) within 3–8 days.

We obtained about 200 seedlings in T_1 from which we selected about sixty (the smallest and those that had somewhat deformed leaves) and transplanted them into small pots. Some died at various stages of development, only thirty-six reaching maturity. The morphological appearance of six plants, 801/1, 801/2, 801/7, 801/8, 801/10 and 801/20, was strikingly different from that of the other thirty and of the controls,



Text-fig. 1. Leaves from four extreme variants and on the right one leaf from *N. tabacum* var. *macrophylla* (the original form).

the latter thirty plants being like the controls of normal *N. tabacum* var. *macrophylla*. Six of these thirty plants had completely normal meiosis and formed about 96–99·5% of viable pollen grains, while the others had various kinds of irregularities in meiosis and different percentages (15–75) of abortive pollen. Details of the meiosis and the percentages of abortive pollen are given in Table I.

N. tabacum var. *macrophylla* has large broad leaves (Text-fig. 1) with intermediate basis and carmine-red flowers (Pl. XVIII, fig. 1). Plant 801/1 had somewhat narrower leaves with small petioles, longer internodes, and elongated flowers of dark red colour. Plant 801/2 had sessile broader leaves, stunted growth, broader but shorter flower tubes and dark red (carmine) corolla colour. Plant 801/7 was dwarf with somewhat elongated petioles; flowers *macrophylla*-like, but lighter in colour. Plant

TABLE I

Cytological characteristics of the T_1 plants, grown in the greenhouse

Plant no.	Somatic chromosome no.	Meiosis: Normal meiosis (*n*)	Meiosis: Univalents	Meiosis: Multivalents	Meiosis: Fragments	Meiosis: Chromatin bridges	Meiosis: Abnormal distribution of the chromosomes during the first meiosis	Tetrad stage	% of viable pollen grains	% of abnormally large pollen grains formed
801/1	50–51	—	1–3	1–2	Rarely	Occasionally	Abnormal	m., d., tr., tetr., pol.	25	3
801/2	52	—	2–4	1–2	—	Occasionally	Abnormal	m., d., tr., tetr., pol.	30	5
801/3	48	*n*	—	—	—	—	—	tetr.	98	—
801/4	—	*n*	—	—	—	—	—	tetr.	99·5	—
801/5	49	—	1	1	—	—	Abnormal	tetr., pol.	60	0·5
801/6	—	*n*	—	—	—	—	—	tetr.	96	—
801/7	50 ± 1	—	2–3	1	Rarely	Occasionally	Abnormal	m., d., tr., tetr., pol.	28	3
801/8	52	—	2–4	1–2	Often	Often	Abnormal	d., tetr., pol.	36	1
801/9	48	—	—	1	Rarely	Rarely	Abnormal	tetr., pol.	80	—
801/10	48 + 1 fr.	—	1	1	Yes	—	Abnormal	tetr., pol.	60	—
801/11	—	—	1	—	—	—	Abnormal	tetr.	88	—
801/12	47 + 1 fr.	—	2	—	Often	—	Abnormal	tetr., pol.	65	—
801/13	—	—	—	1	Rarely	—	Abnormal	tetr.	90	—
801/14	—	—	—	1	—	—	Abnormal	tetr.	88	—
801/15	—	*n*	—	—	—	—	—	—	97	—
801/16	—	—	1	—	—	—	Abnormal	tetr., pol.	88	—
801/17	—	—	—	1	—	Rarely	Abnormal	tetr., pol.	87	—
801/18	48	—	2	—	—	—	Abnormal	tetr., pol.	85	—
801/19	—	—	1	1	—	—	Abnormal	tetr., pol.	92	—
801/20	49	—	1	1	Rarely	—	Abnormal	tetr., pol.	55	—
801/21	—	—	1	—	—	—	Abnormal	tetr., pol.	60	—
801/22	48 + 1 fr.	—	1	1	Yes	Rarely	Abnormal	tetr., pol.	50	—
801/23	—	*n*	—	—	—	—	—	tetr.	87	—
801/24	48 + 1 fr.	—	1–3	1	—	Rarely	Abnormal	tetr., pol.	58	—
801/25	48	—	—	—	Rarely	Rarely	—	d., tetr.	86	0·8
801/26	—	—	1–2	1	—	—	—	tetr.	85	—
801/27	48	—	—	—	—	—	—	—	—	—
801/28	—	—	—	1	—	—	—	tetr.	90	—
801/29	—	—	—	1	—	Rarely	—	tetr.	88	—
801/30	—	*n*	—	—	—	—	Abnormal	tetr.	90	—
801/31	—	—	—	1	Rarely	—	Abnormal	tetr., pol.	87	—
801/32	—	—	—	1	—	—	Abnormal	tetr.	90	—
801/33	48	—	—	—	—	—	—	tetr.	79	—
801/34	48	—	—	—	—	—	—	tetr.	92	—
801/35	—	—	—	—	—	—	Abnormal	tetr., pol.	86	—
801/36	48	—	—	—	—	—	—	tetr.	87	—

m. = monads, d. = dyads, tr. = triads, tetr. = tetrads, pol. = polyads.

801/8 had corrugated sessile leaves and larger dark pink flowers. Plant 801/10 had deformed leaves with exceedingly short petioles and small red flowers reminding those of the variety *N. tabacum* var. *sanguinea*. Plant 801/20 had relatively small, somewhat petiolated leaves and lighter flowers. It was smaller than the others but somewhat larger than the dwarf one.

The data given in Table I show that a series of aneuploid plants was produced in T_1, some having even fragments as a sequence of chromosome rearrangements. The formation of chromatin bridges, no matter how rare, also indicates that chromosome rearrangements (inversions) have taken place. Some of the polyvalents, especially quadrivalents in plants with $2n=48$ and $2n=49$, could be also interpreted by postulating chromosome dislocations.

Monads, dyads, and triads resulted from restitution nuclei. They gave rise further to abnormally large pollen grains.

Meiosis in some of the plants, as for example in nos. 801/33, 801/34, 801/35 and 801/36, was not thoroughly investigated, but these plants, as well as all others, having about 15% and more than 15% of abortive pollen grains, had lagging chromosomes during the first as well as during the second divisions.

Fertility of T_1 plants was closely correlated with the percentage of viable pollen grains. Plant 801/1 set the smallest amount of seeds per capsule, while all of the plants having about 90% and more than 90% of viable pollen were partially or highly fertile when self-pollinated.

In order to obtain plants with increased cytological abnormalities which might give rise further to new variations the more abnormal T_1 plants were crossed, and from them a T_2 generation was raised.

Morphology and cytology of T_2 plants

T_2 families consisted of plants produced by selfing T_1 plants and by crossing various plants of T_1. The latter only were more thoroughly studied and subsequently propagated further. In this T_2 generation a series of variants appeared. Amongst *macrophylla* types variants were obtained reminiscent of already existing varieties of the species *Nicotiana tabacum*. Plants with new combinations of characters and even with new characters were also obtained. There were dwarf plants as well as some with deformed organs (leaves, corolla, anthers, etc.). Details of T_2 plants whose progeny was further studied are given in Table II. They all differed morphologically from the original parental form

N. tabacum var. *macrophylla*, in respect to one or several characters. (*Leaves*: elongated, narrow, broad, petiolated, sessile, etc.; *flowers*: shorter or longer, narrower or broader flower tube, larger or smaller opening of the corolla with lighter or darker colour, the position of the style and anthers in respect to the opening of the corolla; the size of the plant, etc.) Most of the T_2 plants resembling the original *N. tabacum* var. *macrophylla* were fully fertile or almost so; some of the variants were also highly

TABLE II

Cytological characteristics of some T_2 plants

No.	Plant no.	Somatic chromosome nos.	Meiosis
1	S01/1/20	—	Irregular meiosis, univalents and multivalents, laggards, occasionally fragments, only in two instances chromatin bridges
2	S01/1/24	52–53	Irregular meiosis, univalents, multivalents and laggards, one fragment regularly appeared
3	S01/1/25	48	Slightly irregular meiosis, one quadrivalent, occasionally laggards
4	S01/1/26	50–51	Heteromorphic pair, univalents, trivalents and quadrivalents, rarely chromatin bridges
5	S01/2/20	49–50	Univalents, multivalents, laggards
6	S01/2/21	50	Univalents, multivalents, laggards, occasionally one chromatin bridge and one fragment
7	S01/5/25	—	Slight irregularities, laggards
8	S01/5/26	—	Univalents, laggards, rarely trivalent
9	S01/5/27	*ca.* 51	Univalents, multivalents, rarely fragments and chromatin bridges
10	S01/7/22	48 + fr.	Heteromorphic bivalent, univalent, polyvalent, fragment, laggards
11	S01/7/23	48–49	Univalent, multivalent, laggards
12	S01/8/23	*ca.* 50	Univalents, multivalents, fragments and chromatin bridges
13	S01/10/23	48 (+1?)	Abnormal meiosis (it was not thoroughly studied)
14	S01/10/24	50 (+1?)	Univalents, multivalents, laggards
15	S01/10/25	—	Heteromorphic pair, univalent, fragment, laggards
16	S01/20/20	49	Univalent, trivalent, laggards
17	S01/20/23	48	Quadrivalent, rarely laggards
18	*N. tabacum* var. *macrophylla*	48	Normal meiosis

fertile, while the majority of the new variants and several *macrophylla* types were partially fertile. One dwarf and one with deformed flowers, the anthers of which did not open when the pollen grains were ripe, were self-sterile. They set a few seeds when they were artificially self-pollinated with a large amount of pollen. All seventeen T_2 plants given in Table II were partially fertile, some setting more, others fewer seeds.

A T_3 generation was grown from seeds obtained by selfing the plants recorded in Table II. All precautions were taken for avoiding cross-

pollination (except for the plants 801/5/25, 801/5/26, and 801/5/27, in which it might be possible, though not very probable, that cross-pollination has taken place).

Morphology and cytology of T_3 and subsequent generations

Large numbers of variants were investigated up to T_5, and several lines were bred and studied up to T_7. Certain morphological results together with the chromosome numbers of some aberrant plants are summarized in Tables III–IX. Morphological appearance of the leaf and flower of the original variety *N. tabacum macrophylla* are given in Text-fig. 1 and in Pl. XVIII, fig, 1, together with leaves and flowers of some extreme temperature variants. We shall point out here that *N. tabacum* ($n=24$) is an amphidiploid of *N. silvestris* ($n=12$) and *N. tomentosiformis* ($n=12$), as suggested by Clausen (1928) and synthetically produced by Kostoff (1938*b*). This explains the appearance of a series of viable chromosome aberrants, having characters like those of the ancestral species. It may also be mentioned here that F_1 hybrids *N. silvestris* × *N. tomentosiformis* usually formed 0–5 bivalents in the pollen mother cell during the first meiosis (Text-fig. 6). This indicates that some of the single chromosomes in the monosomics, trisomics and tetrasomics may occasionally conjugate (as indeed they do) with the paired ones, exchanging parts and giving rise to new variants.

Details of the progeny of seventeen T_2 plants (i.e. T_3 generation) are given in Table III.

N. tabacum var. *macrophylla* has almost sessile leaves, i.e leaves with extremely short petioles, while *N. silvestris* has sessile leaves. The variants of T_3, T_4, T_5, T_6 and T_7 were roughly divided into three groups in respect to the shape of the leaf basis, namely, into plants with: (1) sessile leaves, (2) petiolate leaves, and (3) intermediate basis, the latter resembling those of *N. tabacum macrophylla*. The degree of the expression of the characters "sessile" and "petiolate" varied greatly (cf. Tables III, VI and VII, and Text-fig. 1). There were variants with sessile leaves, like those of *N. silvestris*, as well as some with long naked petioles, like those of *N. tabacum* var. *fruticosa*.

Each T_3 family segregated in respect of the basal shape of the leaves, giving various ratios. These ratios cannot be evaluated in a strict Mendelian sense, because the T_2 plants were partially sterile, which means that many types of gamete have not participated in the fertilization process, and that a large number of zygotes has been eliminated.

TABLE III

Morphological characteristics and chromosome numbers of the plants in T_2 generation of seventeen families

No.	Families	Plants transplanted	Plants died	Slow-growing plants	Leaf basis: Sessile	Leaf basis: Intermediate	Leaf basis: Petiolate	Extreme variations in leaf shape: *N. silvatris*-like leaves	Extreme variations in leaf shape: Narrow leaves	Extreme variations in leaf shape: Deformed leaves	Plants that flowered	Flower (corolla) colour: Red	Flower (corolla) colour: Pink	Flower (corolla) colour: Pale pink	Flower (corolla) colour: Variegated	Extreme variations in flower shape: Anthers and styles above the corolla	Extreme variations in flower shape: Anthers below the styles	Extreme variations in flower shape: Narrow flowers	Extreme variations in flower shape: Small flowers	Extreme variations in flower shape: Deformed flowers	Plants reaching maturity (of seeds)	Fertility: Fully fertile (× × × ×)	Fertility: Various degrees of fertility (× × ×)	Fertility: Various degrees of fertility (× ×)	Fertility: Various degrees of fertility (×)	Fertility: Sterile	Somatic chromosome numbers: 47	48	49	50	49+1 fr.
1	*N. tabacum* var. *macrophylla*	45	3	1	—	41	—	—	—	—	40	40	—	—	—	—	—	—	—	—	40	40	—	—	—	—	—	—	—	—	—
2	801/1/20	130	20	19	36	28	20	—	—	1	88	68	28	2	—	6	—	2	—	2	73	18	7	33	14	2	3	1	1	—	—
3	801/1/24	45	10	12	8	7	8	—	—	—	22	15	6	1	—	7	—	—	—	—	21	10	4	2	4	1	—	3	1	1	—
4	801/1/25	92	13	6	21	39	14	3	—	—	53	45	21	7	—	6	—	14	—	—	73	6	10	46	9	2	1	4	1	1	—
5	801/1/20	110	1	7	22	26	31	—	12	4	86	60	25	2	—	6	8	13	—	2	74	6	4	41	21	2	1	4	1	1	1
6	801/2/20	91	10	19	7	50	5	—	2	9	52	45	6	1	—	—	—	1	—	1	51	5	10	32	4	—	—	1	2	1	—
7	801/2/21	105	9	17	1	76	2	—	—	—	73	69	2	2	—	—	—	6	—	—	66	3	14	43	6	—	—	3	—	—	—
8	801/5/25	72	11	13	15	24	9	1	—	—	44	25	14	1	4	—	—	—	—	—	43	10	9	16	11	—	2	1	—	—	—
9	801/5/20	48	11	8	12	10	7	—	—	1	29	17	10	1	1	—	—	1	—	—	24	10	4	9	1	—	—	—	—	—	—
10	801/6/27	99	13	13	40	24	9	—	1	3	67	47	17	2	1	15	—	8	—	4	60	16	9	16	19	—	—	2	—	—	—
11	801/7/22	92	6	12	9	16	19	—	2	3	42	29	10	4	—	—	1	4	—	—	41	10	5	18	8	—	—	—	—	—	—
12	801/7/23	88	20	17	21	18	9	—	—	—	36	21	13	2	—	2	—	2	—	9	33	1	20	11	1	—	—	1	—	—	—
13	801/8/24	143	15	24	40	57	17	—	1	3	104	70	24	4	—	—	—	3	—	—	100	15	13	61	9	—	—	2	2	—	—
14	801/10/23	108	32	16	16	29	20	—	1	2	76	50	24	2	—	—	2	4	—	3	70	21	7	31	11	—	—	—	—	—	—
15	801/10/24	107	11	28	23	28	17	—	1	5	46	28	15	2	—	—	2	2	—	5	33	2	2	24	6	—	2	3	2	—	—
16	801/10/25	108	13	26	26	31	10	—	2	6	48	35	14	1	—	2	1	3	—	8	38	14	1	10	12	1	—	5	2	—	—
17	801/20/20	145	12	18	14	45	25	2	—	3	102	62	28	2	—	1	—	4	—	3	92	21	23	24	8	6	—	5	2	—	—
18	801/20/23	57	8	7	20	7	15	—	—	—	37	21	10	—	—	—	1	—	—	—	36	7	8	15	10	6	—	—	—	—	—

TABLE VI

Morphological characteristics and chromosome numbers of the temperature variants in T_1 generation of seventeen families

No.	Families	Plants transplanted	Plants died	Slow growing plants	Leaf basis: Sessile	Leaf basis: Intermediate	Leaf basis: Petiolate	Extreme variations in leaf shape: *N. silvatris*-like leaves	Extreme variations in leaf shape: Narrow leaves	Extreme variations in leaf shape: Deformed leaves	Plants that flowered	Flower (corolla) colour: Red	Flower (corolla) colour: Pink	Flower (corolla) colour: Pale pink	Extreme variations in flower shape: Anthers and styles above the corolla	Extreme variations in flower shape: Anthers below the styles	Extreme variations in flower shape: Narrow flowers	Extreme variations in flower shape: Small flowers	Extreme variations in flower shape: Deformed flowers	Plants reaching maturity (of seeds)	Fertility: Fully fertile (× × × ×)	Fertility: Various degrees of fertility (× × ×)	Fertility: Various degrees of fertility (× ×)	Fertility: Various degrees of fertility (×)	Fertility: Sterile	Somatic chromosome numbers: 47	48	49	50	51	48+1 fr.	48+2 fr.
1	*N. tabacum* var. *macrophylla*	44	2	—	—	42	—	—	—	—	42	42	—	—	—	—	—	—	—	42	42	—	—	—	—	—	—	—	—	—	—	—
2	801/10/24	43	3	8	14	13	5	—	2	—	31	20	9	2	1	2	1	—	—	29	20	—	—	2	11	16	1	2	2	—	—	—	—
3	801/2/24	39	1	1	20	8	—	—	—	—	27	—	26	1	2	—	—	—	—	29	20	—	2	15	2	7	2	2	1	—	—	—	—
4	801/1/23/21	72	4	4	30	25	10	—	—	5	59	33	22	4	0	2	1	3	3	64	—	—	14	28	12	—	—	2	—	—	—	—
5	801/1/20/18	26	2	—	22	2	—	—	—	1	18	—	18	—	—	—	1	—	—	18	—	—	3	8	7	—	—	—	—	1	—	—
6	801/1/24/28	44	4	1	19	14	6	—	1	—	36	33	3	—	12	—	—	1	1	33	8	2	4	14	—	—	1	1	2	1	1	—
7	801/1/25/1	89	8	5	6	18	52	—	—	1	76	72	4	—	—	1	—	—	—	69	—	3	20	27	9	—	—	4	1	1	—	—	—
8	801/1/25/18	49	1	—	39	9	—	35	—	—	43	—	3	40	—	—	—	—	—	43	—	—	17	19	7	—	—	2	1	1	—	—	—
9	801/1/25/21	46	3	1	10	20	12	—	—	—	41	35	6	—	—	—	—	—	—	38	—	—	18	18	2	—	—	—	—	—	—	—	—
10	801/1/20/10	22	3	2	—	15	2	—	—	—	17	—	15	2	—	—	—	—	—	15	—	—	—	3	4	1	1	1	—	—	—	—	—
11	801/1/20/18	43	0	6	10	15	7	—	—	—	31	—	31	—	—	—	—	29	2	24	—	—	6	10	3	1	1	—	—	—	—	—	—
12	801/1/20/23	32	3	1	—	—	28	—	—	—	27	27	—	—	—	—	—	27	—	27	—	—	5	16	0	—	—	2	—	—	2	—	—
13	801/2/21/5	46	2	—	—	44	—	—	—	—	24	24	—	—	—	—	—	23	—	23	—	3	0	9	8	—	—	3	—	—	—	—	—
14	801/3/23/2	41	0	3	22	10	—	—	—	—	26	1	24	1	—	—	—	—	—	25	—	—	4	14	7	—	—	1	1	—	—	—	—
15	801/3/23/14	45	2	—	9	30	4	—	—	—	42	42	—	—	32	—	—	—	—	40	—	1	2	30	7	—	—	—	1	2	—	—	—
16	801/3/23/2	23	3	2	18	—	—	—	—	—	18	—	18	—	—	—	—	—	—	13	—	—	3	6	4	—	—	2	—	—	—	—	—
17	801/20/20/12	92	2	3	87	3	—	76	—	—	84	—	81	—	—	—	—	—	—	70	—	2	20	41	17	—	—	2	1	—	1	—	—
18	801/20/20/3	83	5	3	60	15	—	52	—	—	74	1	64	9	—	—	—	—	—	60	—	0	22	22	16	1	—	1	—	—	—	—	—

TABLE IX

Morphological characters and chromosome numbers of sixteen variants and back-crosses

No.	Families	Plants transplanted	Plants died	Slow growing plants	Leaf basis: Sessile	Leaf basis: Intermediate	Leaf basis: Petiolate	Plants that flowered	Flower (corolla) colour: Red	Flower (corolla) colour: Pink	Flower (corolla) colour: Pale pink	Anthers and styles above the corolla	Narrow flowers	Plants reaching maturity (of seeds)	Fertility: Fully fertile (× × × ×)	Fertility: Various degrees of fertility (× × ×)	Fertility: Various degrees of fertility (× ×)	Fertility: Various degrees of fertility (×)	Fertility: Sterile	Somatic chromosome numbers: 47	48	49	50	48+1 fr.	48+2 fr.	48+1 fr.
1	*N. tabacum* var. *macrophylla*	25	0	5	—	14	—	12	12	—	—	—	—	12	12	—	—	—	—	—	—	—	—	—	—	—
2	801/7/23-3	36	7	10	16	—	—	18	18	—	—	13	—	14	—	4	6	3	1	—	1	1	2	—	—	—
3	801/20/20-2	24	5	7	3	7	2	12	8	3	1	—	1	12	—	2	6	3	2	—	—	1	2	1	—	—
4	801/10/24-3	23	7	2	14	—	—	5	4	1	—	—	—	4	—	1	2	1	—	—	—	—	—	—	—	—
5	801/10/24-23	25	3	5	12	7	—	5	—	—	—	—	—	4	—	1	2	1	—	—	—	1	1	—	—	—
6	801/10/24-23 × *N. tabacum* var. *macrophylla*	24	5	6	10	8	1	12	—	—	—	—	—	11	—	2	0	1	—	—	—	—	—	—	—	—
7	801/1/20-21-3	74	14	13	37	2	—	29	—	—	—	—	—	22	1	0	13	2	1	—	—	—	—	—	—	—
8	801/1/20/21-3 × *N. tabacum* var. *macrophylla*	48	10	8	11	19	—	25	—	—	—	—	—	10	—	0	10	—	—	—	—	—	—	—	—	—
9	801/1/20-28-2	25	3	4	9	9	—	10	—	—	5	—	—	9	—	0	4	2	1	—	—	1	—	—	—	—
10	801/1/25-1-3	48	13	12	—	—	23	14	4	1	—	—	—	10	—	4	3	1	—	—	—	—	—	—	—	—
11	801/1/25-1-3 × *N. tabacum* var. *macrophylla*	25	11	6	—	—	8	6	—	—	—	—	—	6	—	—	5	1	—	1	2	—	—	—	—	—
12	801/1/25-18-10	42	6	12	25	—	—	—	4	16	—	—	—	16	—	0	7	—	—	—	—	1	—	—	—	—
13	801/1/25-18-10 × *N. tabacum* var. *macrophylla*	46	12	8	6	13	3	16	—	—	—	—	—	14	—	2	8	3	1	—	—	—	—	—	—	—
14	801/1/25-18-44	42	14	12	13	3	—	—	3	7	—	—	—	9	—	2	3	3	1	—	2	1	—	—	—	—
15	801/1/25-18-44 × *N. tabacum* var. *macrophylla*	42	13	11	13	2	—	11	1	—	—	9	8	9	—	2	4	3	1	—	—	1	—	—	—	—
16	801/1/20-22-10	23	9	7	—	—	—	9	—	—	—	—	7	6	—	4	1	1	—	—	1	—	—	1	—	—
17	801/1/20-22-10 × *N. tabacum* var. *macrophylla*	25	6	7	—	—	12	7	—	—	—	—	—	6	—	—	—	—	—	—	—	—	—	—	—	—

T_3 plants showed varying degrees of fertility. They were divided into five groups in respect of the number of the seeds they set when grown in the field: (1) fully fertile plants setting about 1200 seeds per capsule ($\times \times \times \times$), (2) highly fertile plants setting about 600 seeds per capsule ($\times \times \times$), (3) partially fertile plants, setting about 150 seeds per capsule ($\times \times$), (4) highly sterile ones setting less than fifty seeds per capsule ($\times$), and (5) completely sterile ($-$) setting no seeds. Plants of intermediate fertility were placed in the class to which they approximated most closely.

T_4 and T_5 plants were also classified into these five fertility groups (Tables VI and IX).

Temperature variants differed from each other in respect of the size of the plants, habit of growth and flower shapes and colours. It should be mentioned here that *N. silvestris*, one of the ancestors of *N. tabacum*, has long white flowers, of which the upper part of the styles and of the longest anthers reach the opening of the floral tube. *N. tomentosiformis*, on the other hand, the other ancestor of *N. tabacum*, has relatively short pink reddish flowers, the anthers and the styles of which project several millimetres (4–7) above the opening of the corolla. The flowers of the temperature variants had various corolla colours, flower size and shape as well as various lengths of anthers and styles. Some were dark red colour, others almost white, while a large number had pink flowers of various intensity (cf. Plate figures and Tables III, VI and IX).

The position of the stigma and the anthers with respect to the opening of the corolla varied greatly; in some the anthers and styles projected as far as the opening of flower tubes (as in *N. tabacum* var. *macrophylla* and in *N. silvestris*), in others they projected somewhat above the opening (as in *N. tomentosiformis*) (cf. Pl. XVIII and Tables III, VI and IX). The segregation ratios for flower colour in T_3, T_4 and T_5 are given in Tables III, VI and IX. These data cannot be used for determining the mode of inheritance of flower colour, since the parental plants had abnormal meiosis and were not fully fertile, but there is a definite tendency for certain variants with pink flowers to segregate into red, pink and light pink.

Partially fertile temperature variants gave rise occasionally to plants with deformed leaves or/and flowers. The morphological appearance of the variants was not necessarily correlated with fertility, since there were *macrophylla* types with reduced fertility as well as highly fertile new types which produced fully fertile variants in subsequent generations (up to T_7). The plants considered in Tables III, VI and IX were chiefly

progenies of the most extreme variants. They usually showed marked cytological anomalies, which naturally conditioned reduced fertility.

The degree of fertility of some of the variants of the subsequent generations was not always necessarily correlated with the percentage of the viable pollen grains, since there were variants that had almost normal pollen grains, though the number of seeds per capsule was greatly reduced.

Some of the variants were sterile when self-pollinated; they set, however, as many seeds as *N. tabacum macrophylla*, when pollinated with pollen of this variety (i.e. *ca.* 1200–1500 per capsule). These observations suggest that the velocity of the pollen-tube growth of some variants was significantly reduced, though an alternative cause is also possible, viz. elongation of the styles, as was the case with some variants.

With regard to the size of T_3 and T_4 variants (Tables IV and VII) as compared with that of the original form (*macrophylla*) the following two statements can be made: (1) the plants of a series of families were more variable in size than those of the original form, and (2) the size of single plants as well as the average size of a series of families were much larger than *N. tabacum macrophylla*. There were also single plants and average values of whole families that were smaller than those of *N. tabacum macrophylla*.

The number of the leaves per plant is a character of great agricultural importance. Grown under the same conditions *N. tabacum* var. *macrophylla* averaged twenty leaves per plant as against twenty-six for the T_3 family 801/1/24. There were, however, families with a much smaller average number, namely fifteen (801/1/26) and sixteen (801/8/23). Extreme plus variants of *macrophylla* had 24–25 leaves per plant, while some of the temperature variants had as many as 50–51 leaves per plant (801/2/20) (Table V).

T_4 temperature variants behaved in a similar way (Table VIII). In the same environmental conditions in which *macrophylla* plants had nineteen leaves per plant, T_4 family 801/1/24/28 had twenty-seven leaves per plant, single plants of which had 39–40 leaves. Some T_4 families had more variable numbers of leaves per plant than *macrophylla*, while a few families had less variable numbers.

In T_3, T_4, T_5, T_6 and T_7 we found variants with 47, 48, 49, 50 and 51 somatic chromosomes. Some of them had one, or more than one, fragment, i.e. exceedingly small chromosomes (cf. Tables III, VI and IX and Pl. XIX).

Some of the variants were highly constant in subsequent generations,

TABLE IV

Plant size in centimetres of the T_3 generation

No.	Families	31–40	41–50	51–60	61–70	71–80	81–90	91–100	101–110	111–120	121–130	131–140	141–150	151–160	161–170	171–180	181–190	191–260	*n*	*M*
1	*N. tabacum* var. *macrophylla*	—	2	3	4	5	4	9	10	1	—	—	—	—	—	—	—	—	38	85·8
2	801/1/20	—	2	—	4	6	5	10	15	17	21	3	5	—	—	—	—	—	88	106·1
3	801/1/24	—	—	—	1	1	1	—	—	4	4	4	3	1	1	—	—	2	22	126·3
4	801/1/25	—	—	1	4	1	—	5	4	13	14	12	9	6	3	1	—	—	73	124·0
5	801/1/26	—	6	6	8	7	12	14	14	9	2	4	3	1	—	—	—	—	86	90·2
6	801/2/20	1	1	4	6	5	10	8	7	6	1	3	—	—	—	—	—	—	52	87·6
7	801/2/21	2	2	1	6	5	12	19	12	8	6	—	—	—	—	—	—	—	73	90·8
8	801/8/23	—	1	1	—	1	3	8	5	12	8	2	3	—	—	—	—	—	44	107·0
9	801/5/26	—	—	—	1	1	1	7	3	5	7	2	2	—	—	—	—	—	29	109·0
10	801/5/27	—	—	1	2	5	5	2	13	11	13	7	4	3	1	—	—	—	67	112·1
11	801/7/22	—	—	2	3	10	7	6	4	3	3	2	—	1	1	—	—	—	42	86·0
12	801/7/23	—	—	6	1	8	7	6	2	2	1	1	1	—	1	—	—	—	36	86·0
13	801/8/23	1	1	4	6	5	15	22	8	19	12	7	3	1	—	—	—	—	104	81·8
14	801/10/23	—	2	6	2	3	12	19	13	12	4	2	1	—	—	—	—	—	76	97·6
15	801/10/24	—	—	3	3	4	7	8	9	4	6	1	—	—	—	—	—	—	45	93·3
16	801/10/25	—	5	7	2	6	8	6	10	2	1	—	1	—	—	—	—	—	48	82
17	801/20/20	1	4	6	8	11	17	17	16	10	6	4	2	—	—	—	—	—	102	90·2
18	801/20/23	—	2	1	2	8	3	8	7	1	5	—	1	—	—	—	—	—	38	90·2

TABLE V

Number of leaves per plant in T_3 generation

No.	Families	6–7	8–9	10–11	12–13	14–15	16–17	18–19	20–21	22–23	24–25	26–27	28–29	30–31	32–33	34–35	36–37	38–39	40–41	42–43	44–45	46–47	48–49	50–51	*n*	*M*
1	*N. tabacum* var. *macrophylla*	—	—	—	—	2	4	6	15	9	2	—	—	—	—	—	—	—	—	—	—	—	—	—	38	20
2	S01/1/20	—	—	—	1	2	6	16	23	13	5	6	6	2	2	1	1	—	3	—	1	1	—	—	88	23
3	S01/1/24	—	—	—	2	1	1	—	1	3	4	2	2	1	2	—	1	1	—	—	—	—	1	—	22	26
4	S01/1/25	—	—	—	2	9	15	16	10	5	6	2	1	1	—	2	2	1	—	1	—	—	—	—	73	21
5	S01/1/26	—	2	10	22	16	14	9	2	1	2	1	1	—	—	2	1	3	—	—	—	—	—	—	86	15
6	S01/2/20	—	—	1	5	13	13	11	3	1	—	1	—	—	1	1	—	—	—	—	—	—	—	2	52	18
7	S01/2/21	—	—	2	5	10	18	16	9	1	—	—	—	—	—	—	1	—	—	1	—	1	—	—	73	18
8	S01/8/23	—	—	1	3	9	8	12	5	2	1	1	1	—	1	—	—	—	—	—	—	—	—	—	44	18
9	S01/5/26	—	—	—	2	1	4	8	4	3	1	1	2	—	—	1	—	—	1	—	—	1	—	—	29	22
10	S01/5/27	—	—	—	—	2	10	12	12	10	8	—	4	2	1	1	1	3	—	1	—	—	—	—	67	22
11	S01/7/22	—	—	1	—	2	8	8	8	3	2	1	2	1	—	1	1	1	2	1	—	—	—	—	42	21
12	S01/7/25	—	—	1	1	7	6	1	3	4	1	4	2	2	2	—	—	2	—	1	—	—	—	—	37	23
13	S01/8/23	1	—	4	3	12	22	19	22	10	3	—	2	1	1	—	2	—	1	—	—	1	—	—	104	16
14	S01/10/23	—	—	—	—	5	7	13	20	9	7	2	8	2	—	—	—	—	—	3	—	—	—	—	76	22
15	S01/10/24	—	—	—	—	3	2	10	6	13	6	1	1	2	1	—	—	—	—	—	—	—	—	—	45	22
16	S01/10/25	—	—	—	2	4	2	11	11	8	1	3	3	—	1	1	—	—	—	—	1	—	—	—	48	21
17	S01/20/20	—	2	1	10	15	18	12	15	11	5	—	5	3	2	1	—	—	1	—	1	—	—	—	102	19
18	S01/20/23	—	1	2	7	12	5	3	1	3	2	—	—	1	—	2	—	1	—	—	—	—	—	—	40	18

while the others segregated, giving rise to plants with characters existing in other varieties in the species *N. tabacum* or with new characters. In the majority of the cases the deviations from the original form and further divergencies could be more readily expressed in "degrees", "sizes" and "numbers", some of which were further heritable (degree of flower colour, ranges between extreme petiolation and sessileness of the leaves, position of anthers and styles, size of the plants, size of the flowers, etc., number of the leaves, etc.).

Some of the fragments, or rather the extremely short chromosomes, resulting from temperature treatments were often regularly transmitted up to T_7, which means that plants with new karyotypes have been produced.

Studying a large number of temperature variants of different generations and families, a series of interesting data was collected throwing light on the nature and behaviour of the experimentally produced variants and indicating that the same processes can also occur in nature.

DESCRIPTION OF SELECTED VARIANTS

We shall describe here (so far as space permits) the cytogenetic behaviour of a series of variants which will serve as a basis for elucidation of their mode of origin and evolutionary significance.

Variant 801/2/21 *p* 3 is a T_3 plant from family 801/2/21. The latter was very uniform and morphologically was like *N. tabacum macrophylla* (habit of growth, flower shape and colour, leaves), the flowers being only somewhat smaller than those of *macrophylla*. The majority of the plants had reduced fertility. Plant 3 of this family had 48 somatic chromosomes and was partially fertile ($\times\times$). The large chromosome number (48) presents great difficulties for identifying minute chromosome dislocations in comparing the karyotype of 801/2/21 *p* 3 with the karyotype of *macrophylla*. But in studying the procedure of the meiotic processes in this plant, a trivalent chromosome group and one univalent were occasionally found. One chromosome pair was heteromorphic, one chromosome being longer than the other. One, and occasionally two laggards have been found in some pollen mother cells during the first meiosis. The second division proceeded normally. The plant formed about 97·5% viable pollen but was partially sterile ($\times\times$). The cytological behaviour of this plant indicates that reduced fertility is probably due to an unequal exchange of parts, or to a simple translocation which most probably conditions a retardation of the pollen-tube growth. The progeny of this plant were also *macrophylla*-like, but again

TABLE VII

Plant size in centimetres of T_4 generation

No.	Families	31–40	41–50	51–60	61–70	71–80	81–90	91–100	101–110	111–120	121–130	131–140	141–150	151–160	161–170	171–180	181–190	191–200	*n*	*M*
1	*N. tabacum* var. *macrophylla*	—	—	3	10	18	5	3	2	1	—	—	—	—	—	—	—	—	42	81·6
2	S01/10/24	—	2	5	5	5	9	3	1	—	1	—	—	—	—	—	—	—	31	76·5
3	S01/1/20/1	—	—	2	4	6	—	7	1	3	2	2	—	—	—	—	—	—	27	89·0
4	S01/1/20/21	—	—	2	5	3	9	12	8	3	5	2	1	3	3	2	1	—	59	100·1
5	S01/1/20/18	—	1	—	2	—	2	5	2	1	2	2	—	—	1	—	—	—	18	100·8
6	S01/1/24/28	—	—	—	—	2	1	6	5	5	4	4	4	3	1	—	1	—	36	116·2
7	S01/1/25/1	—	1	1	3	5	3	13	4	9	12	12	7	2	2	2	—	—	76	113·5
8	S01/1/25/18	—	—	1	2	1	3	2	10	3	2	9	3	4	3	—	—	—	43	117·0
9	S01/1/25/21	4	2	7	4	2	8	1	5	4	2	1	—	1	—	—	—	—	41	79·8
10	S01/1/26/10	—	1	3	1	2	1	1	3	—	2	2	1	—	—	—	—	—	17	92·1
11	S01/1/26/18	1	3	6	5	—	5	2	4	3	—	1	1	—	—	—	—	—	31	78·8
12	S01/1/26/22	—	2	2	2	5	3	2	7	4	—	—	—	—	—	—	—	—	27	86·1
13	S01/2/21/5	—	—	5	2	3	2	3	4	2	1	1	1	—	—	—	—	—	24	90·1
14	S01/8/23/2	—	1	3	3	3	3	2	1	1	4	1	2	2	—	—	—	—	26	95·9
15	S01/8/23/11	—	—	1	2	5	3	4	2	3	2	4	5	2	6	—	2	1	42	119·6
16	S01/8/23/2	—	—	1	2	1	2	1	—	1	1	2	—	—	—	1	—	—	12	97·6
17	S01/20/20/12	—	5	7	13	8	12	10	7	6	4	4	2	2	1	—	—	—	81	87·8
18	S01/20/20/3	1	4	7	7	6	10	6	13	9	1	—	3	1	—	—	1	—	69	93·2

TABLE VIII

Number of leaves per plant of T_4 generation

No.	Families	6–7	8–9	10–11	12–13	14–15	16–17	18–19	20–21	22–23	24–25	26–27	28–29	30–31	32–33	34–35	36–37	38–39	40–41	42–43	*n*	*M*
1	*N. tabacum* var. *macrophylla*	—	—	—	1	2	6	19	8	4	2	—	—	—	—	—	—	—	—	—	42	19
2	801/10/24	—	—	—	—	—	3	5	9	6	7	1	—	—	—	—	—	—	—	—	31	21
3	801/1/20/1	—	—	—	—	—	1	6	5	9	3	4	—	—	—	—	—	—	—	—	27	22
4	801/1/20/21	—	—	—	—	—	3	9	21	15	9	2	—	—	—	—	—	—	—	—	59	21
5	801/1/20/18	—	—	—	—	—	1	3	2	4	4	3	1	—	—	—	—	—	—	—	18	23
6	801/1/24/28	—	—	—	—	—	1	2	5	6	8	2	2	2	3	1	2	1	1	—	36	27
7	801/1/25/21	—	—	—	—	—	1	3	9	17	26	13	3	3	1	—	—	—	—	—	76	25
8	801/1/25/18	—	—	—	—	—	2	6	8	18	11	3	4	1	—	—	—	—	—	—	53	24
9	801/1/35/21	—	—	—	2	3	11	10	10	3	2	—	—	—	—	—	—	—	—	—	41	19
10	801/1/26/10	—	—	—	—	—	—	5	8	3	1	—	—	—	—	—	—	—	—	—	17	20
11	801/1/26/18	—	1	—	3	7	2	6	8	2	1	—	1	—	—	—	—	—	—	—	31	18
12	801/1/26/22	—	1	—	2	7	8	8	—	1	—	—	—	—	—	—	—	—	—	—	27	16
13	801/2/21/5	—	—	2	2	5	4	6	2	3	—	—	—	—	—	—	—	—	—	—	24	17
14	801/3/23/2	—	—	—	2	3	2	2	7	3	5	1	—	1	—	—	—	—	—	—	26	20
15	801/8/23/11	—	—	—	1	2	3	4	7	7	7	6	3	1	2	—	—	—	—	—	43	23
16	801/8/23/2	—	1	—	—	—	—	—	2	4	5	1	—	—	—	—	—	—	—	—	13	23
17	801/20/20/12	—	—	1	4	19	11	13	18	12	3	—	—	—	—	—	—	—	—	—	81	18
18	801/20/20/3	—	—	1	5	9	12	21	11	6	4	2	1	1	1	—	—	—	—	—	74	19

Remark. Plants of T_3 family 801/10/24 were grown together with the families of T_4 generation for comparing their behaviour.

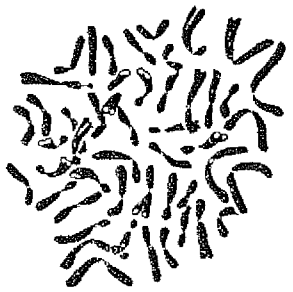
with reduced fertility. Three T_4 plants were studied cytologically. They had 48 somatic chromosomes.

Variant 801/20/20 *p* 2 diverged greatly from the original form, having petiolate leaves with small wings, flower tubes becoming gradually broader and forming a funnel at the upper end; corolla red; tips of the

Text-fig. 2.

Text-fig. 3.

Text-fig. 4.



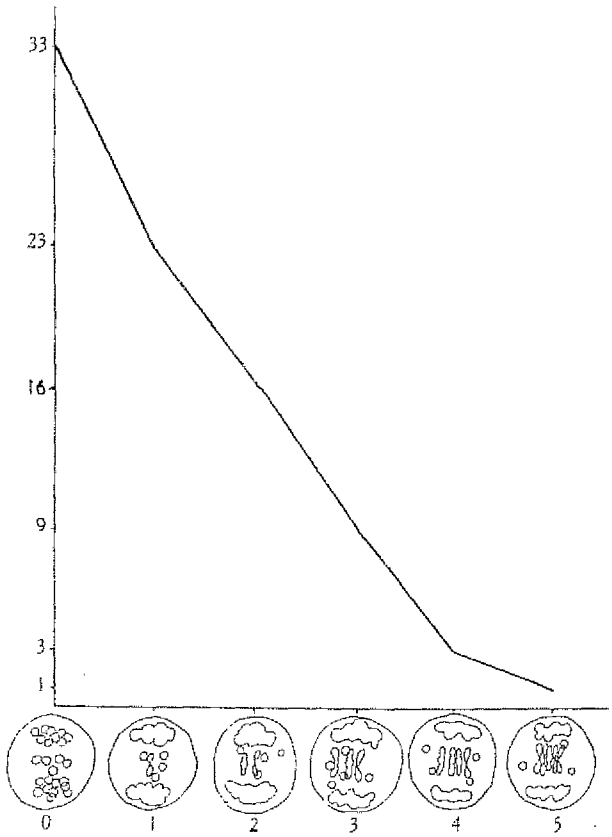
Text-fig. 5.

Text-fig. 2. Somatic plate of variant 801/1/25 *p* 5 having 48 chromosomes.
Text-fig. 3. Somatic plate of variant 801/20/20 *p* 2 having 48 chromosomes.
Text-fig. 4. Somatic plate of variant 801/1/26/18 *p* 27 having 48 chromosomes.
Text-fig. 5. Somatic plate of variant 801/10/24 *p* 2 having 49 chromosomes.

petals with a very small angle; styles reaching the upper end of the floral tubes; anthers situated below the stigma. Although forming about 98% of viable pollen grains, seeds were set only on artificial self-pollination owing to "heterostily". It had 48 somatic chromosomes (Text-fig. 3). During diakinesis a strikingly heteromorphic bivalent was found. Trivalents and univalents (cf. Pl. XIX, fig. 2) were also

occasionally found. Laggards were rarely observed. Second division proceeded normally. The plant set about 600 seeds per capsule when artificially self-pollinated.

Variant 801/1/25 *p* 5 was one of the most extreme forms obtained. Leaves elongated, ovoid, with long petioles, markedly different from



Text-fig. 6. Diagram showing the frequency of the bivalents in F_1 hybrid *Nicotiana silvestris* × *N. tomentosiformis*. Abscissa, number of bivalents; ordinate, number of pollen mother cells studied.

those of *macrophylla*. Calyx and floral tube narrower, but longer than in *macrophylla*, corolla red, but somewhat lighter than in *macrophylla* with yellowish shades, petals very long and somewhat turned down; style projecting above the opening of the tube; anthers situated somewhat below stigma. Looking over the world collection of *N. tabacum* varieties in the Tobacco Institute we have found no variety like this strange type. It formed about 94·2% of viable pollen unequal in size,

but was nevertheless self-sterile. This plant had 48 somatic chromosomes. Meiosis was not studied because the plant was broken at the beginning of the florescence period.

Variant 801/1/26/10 *p* 16. This T_4 plant had *macrophylla*-like leaves, but pink flowers. It had 48 somatic chromosomes, and in both together second metaphases in the pollen mother cells (P.M.C.). In studying the diplotene stage, we found undoubted pairs with unequally long chromosomes. A large chromomere was missing (deficiency) in one of the partners. There were also bivalents that had in some regions morphologically different chromomeres. The heteromorphic pair was also to be distinguished during diakinesis. Trivalents and univalents were also found. The latter appeared as laggards during the first anaphase. Second meiosis proceeded almost normally. The plant formed about 91·5% of viable pollen, but had very low fertility (×).

Variant 801/10/24 *p* 10 had sessile leaves and pink flowers. It was a monosomic having 47 chromosomes. During the first metaphase one univalent (*A*) and 23 bivalents were usually found, but in some P.M.C. one trivalent (*ABB*) and 22 bivalents were found, which means that an unpaired chromosome (*A*) conjugates and forms chiasma (or chiasmata) with the chromosomes of another pair (*BB*), exchanging parts with one of them. The conjugation and chiasma formation between *A* and *B* chromosomes in the trivalent group proceeds between homologous segments of *A* and *B* chromosomes (if such were present) or between heterochromatic regions of the non-homologous chromosomes (*A* and *B*) (cf. Kostoff, 1938*a*). The monosomic plant was partially fertile.

Variant 801/1/26/18 *p* 27 had sessile elongated leaves and narrow flower tubes ending at the top with a funnel-like opening. The flowers were pink and smaller than in *macrophylla* (Pl. XVIII, fig. 9). This variant has 48 chromosomes. It has reorganized chromosomes, since heteromorphic bivalents were found during the early prophase. Trivalents (and possibly some multivalents) were occasionally formed. The plant formed *ca.* 89·5% of viable pollen and was partially fertile.

Variant 801/7/23 *p* 3 had sessile leaves and red, *macrophylla*-like flowers; both anthers and stigmas were projecting several millimetres above the corolla. It had 50 somatic chromosomes, and their behaviour during diakinesis and first metaphase suggested strongly that it was a tetrasomic with some chromosome dislocations (cf. Pl. XIX, figs. 3–6). It formed a variable number of bivalents, univalents, trivalents, and quadrivalents. Heteromorphic pairs were also found during diakinesis and first metaphase, one chromosome being significantly longer than

the other. In counting the numbers of the chromatin bodies during the first metaphase (polar view) the following data were obtained:

The nos. of the chromatin bodies	24	25	26	27	Total
P.M.C.	13	35	11	1	60

These chromatin bodies were bivalents, univalents, trivalents and quadrivalents. The most peculiar phenomenon observed in this plant was the ring trivalent which suggests a conjugation of chromosomes with ends:

$$AB\text{–}BC\text{–}CA, \text{ or } BmA\text{–}AmA\text{–}AmB.$$

An interesting cytological phenomenon observed in this plant is the occurrence of one or two fragments during diakinesis and early metaphase (Pl. XIX, figs. 4, 5). During the anaphases one, and occasionally two chromatin bridges were observed (Pl. XIX, fig. 5). Since fragments have not been found in the somatic plates, and since chromatin bridges are formed during the meiotic anaphases, it seems very probable that the small fragments observed during diakinesis and the first meiotic metaphase resulted from crossing-over in inverted regions and precocious terminalization. The fragments resulting from crossing-over in inverted regions separate precociously so that the bridges formed during the first anaphases are not usually accompanied with fragments lagging on the spindles (Pl. XIX, fig. 6). Precociously separated fragments usually joined the groups of the chromosomes situated at the poles during the first anaphase, and can be easily seen during the subsequent phases of the meiosis. Chromosome distribution during the first meiosis has occurred abnormally; therefore, second metaphases with 23, 24, 25, 26 and 27 chromosomes were found (Pl. XIX, figs. 7, 8). Slight abnormalities were also noticed during the second meiosis. The plant formed about 96% of viable pollen and was partially fertile ($\times\times$). The progeny of this plant had sessile leaves and red flowers. Most of the plants had anthers and stigmas above the opening of the flowers. Those studied cytologically had 48, 49 and 50 chromosomes. Plant 801/7/23/3 p 3 of this family had 48 chromosomes, and its anthers and stigmas reached the opening of the flower tube. It had relatively slight meiotic abnormalities (laggards) and was highly fertile.

Another plant of the same family, namely 801/7/23/3 p 1, had also 48 chromosomes, but its anthers and stigmas projected somewhat above the opening of the floral tubes. Meiosis was relatively regular, occasionally one or two laggards were found. It formed about 95% of viable pollen,

nevertheless, it was partially fertile (× ×), showing lower fertility than a trisomic plant ($2n = 49$) of the same family which had occasionally up to five or six laggards, but was highly fertile (× × ×). The latter plant had sessile leaves, red flowers and anthers and stigmas situated above the openings of the floral tubes.

Two more plants of this family will be considered here, namely 801/7/23/3 *p* 5 and 801/7/23/3 *p* 11, both having anthers and stigmas above the openings of the floral tubes, but the former had 50 somatic chromosomes, the latter 48. Both had laggards during meiosis and were partially fertile.

Variant 801/1/24 *p* 28 had ovoid leaves with *macrophylla*-like basis, the lower part of the flower and corolla colour were *macrophylla*-like, while the corolla was much larger than in *macrophylla*, the ends of the petals being somewhat turned down. Anthers and stigmas projected several millimetres above the corolla.

The somatic chromosome number of this plant was $2n = 49$. Meiosis was somewhat irregular. This plant was not simply a trisomic, because it formed during the first meiotic metaphase 23 bivalents and 3 univalents as well as 23 bivalents and 1 trivalent.

Trivalents often appeared as a closed ring, especially during diakinesis. During this phase, as well as during metaphase, a heteromorphic bivalent appeared with a relatively larger partner. In a few P.M.C., even a quadrivalent was found. Laggards together with one, and in some cells more than one, chromatin bridge were also observed. The latter were occasionally accompanied with small fragments. Fragments were also observed during the second metaphase.

The formation of chromatin bridges and the appearance of second metaphases with $25 + 24$ chromosomes with additional fragment is good evidence for inversion in this variant. The chromosome numbers of the second metaphases varied from 23 up to 26. The plant formed about 95 % viable pollen but was partially fertile (× ×).

Variant 801/10/24 *p* 2 differed from the above, having sessile leaves, red cinnabar flower colour, somewhat narrower opening of the upper end of the floral tube and smaller petals, though it had also 49 somatic chromosomes (Text-fig. 5). In studying the meiosis of this plant (Pl. XIX, fig. 9) one, and sometimes two, univalents were found. It also formed one trivalent and one heteromorphic bivalent. Laggards were also seen during both meioses. Although it formed a relatively high percentage of viable pollen it was partially fertile.

In studying cytologically seven plants of the progeny of plant

801/1/24 *p* 28, the following chromosome numbers were found: (1) 47, (2) 48, (3) 49, (4) two plants, 50, (5) one plant, 51, and (6) one had 48 chromosomes plus 2 fragments (very small chromosomes).

We shall consider here one representative of each of these six types.

Variant 801/1/24/28 *p* 15. Leaves sessile, flowers red, stigma and anthers above the corolla. Chromosome number 47. Meiosis irregular, forming $23^{II}+1^{I}$ and even $22^{II}+1^{III}$. Viable pollen 84%. Fertility low.

Variant 801/1/24/28 *p* 32. Leaves ovoid with intermediate basis that can be rather classified to "petiolate". Pink flowers. Chromosome number 48. Meiosis regular. Laggards were rarely found. It formed usually 24 bivalents and was highly fertile (× × ×).

Variant 801/1/24/28 *p* 14 was a very vigorous plant. Leaves ovoid with intermediate basis. Red flowers. Chromosome number 49. Meiosis (Pl. XIX, figs. 10, 11) resembled in many respects that of the parental plant 801/1/24/28. It formed trivalents and univalents. Second metaphase with more than 49 chromosomes in both plates was occasionally found, resulting from division of univalent chromosomes during the first meiosis (Pl. XIX, fig. 11).

Variant 801/1/24/28 *p* 9. Leaves ovoid, flowers red, much smaller than in *macrophylla*. Anthers and stigmas above the corolla. Chromosome number 50. It formed multivalents and heteromorphic bivalents during the meiosis. It also formed about 82% of viable pollen grains and very small shrunken capsules. Almost normal capsules were produced with an amount of seeds that normal *tabacum* plants form, when it was pollinated with pollen from normal *N. tabacum* plants. Pollen grains formed by this plant have usually more than 24 chromosomes (*n*). Back-cross experiments showed that the pollen tubes of these pollen grains grow slowly and a very small percentage reach the ovary, thus conditioning a low fertility after self-pollination.

Variant 801/1/24/28 *p* 17. Dwarf. Ovoid sessile leaves. Red flowers. Chromosome number 51. It formed a variable number of bivalents, trivalents, tetravalents, and univalents. Occasionally a pentavalent was also found. Heteromorphic bivalents were also observed. Small fragments were seen during diakinesis, metaphase and anaphase (Pl. XIX, figs. 12, 13). Univalent chromosomes, lagging on the spindle during the first meiosis, occasionally undergo longitudinal division. The plant formed about 80% of viable pollen and was self-sterile. Self-sterility of this variant can also be interpreted by slow growth of the pollen tubes, all of them having hyperploid chromosome numbers.

Variant 801/1/24/28 *p* 2. Ovoid leaves with *macrophylla*-like basis. Flowers (Pl. XVIII, fig. 6) dark pink, more slender than in *macrophylla*, with longer calyx; anthers and stigmas situated several millimetres above the corolla. Chromosome number 48 + 2 "fragments". The "fragments" are in reality exceedingly small chromosomes, one having almost median constriction (centromere). It usually formed 24 bivalents during the meiosis, both fragments remaining as univalents. In some P.M.C. only 24 chromatin bodies were found. It was difficult to decide whether the small chromosomes were eliminated during the previous cell divisions, or whether they conjugated with some bivalents (forming trivalents). The plant formed *ca.* 89 % of viable pollen and was partially fertile (× ×).

Five plants of the progeny of variant 801/1/24/28 *p* 2 were studied cytologically. One had 48 chromosomes, two had 48 + 1 fragment, two had 48 + 2 fragments and one had 49 + 1 fragment.

Variant 801/1/24/28/2 *p* 2 had sessile leaves, red flowers and 48 chromosomes. During the first meiosis usually 24 bivalents were seen. Laggards also appeared occasionally. The plant was highly fertile (× × ×).

Variant 801/1/24/28/2 *p* 12 had sessile leaves, red flowers, stigma elevated above the corolla, while the anthers were situated just in the opening of the latter. The chromosome number of this plant was 48 + 1 fragment. The fragment appeared usually as a univalent chromosome during the first meiosis. This variant was highly fertile (× × ×).

Variant 801/1/24/28/2 *p* 1 had leaves with *macrophylla*-like basis, and light red (dark pink) flowers, with both anthers and stigmas projecting above the corolla. The chromosome number of this variant was 48 + 2 fragments. The fragments often occurred as univalent chromosomes during the first meiosis. In some P.M.C. they may remain in the cytoplasm, so that second metaphases with 24 + 24 chromosomes were found. The plant had reduced fertility (× ×).

Variant 801/1/24/28/2 *p* 4 had sessile leaves and red flowers. Anthers and stigmas were situated above the corolla. The chromosome number was 49 + 1 fragment. In studying the meiosis (cf. Pl. XIX, figs. 14–16) we found that a fragment (or rather a small chromosome) appeared usually as a univalent. A trivalent occurred quite often in the shape of a ring (*–ab–bc–ca–*) or as a chain. The small chromosome that appeared as a univalent often split during the first anaphase (Pl. XIX, fig. 15). One or two laggards (sometimes including the small chromosome) occurred during the first, as well as during the second metaphase. Delayed terminalizations in the trivalent group as well as chromatin bridges were also observed.

It has already been mentioned that in T_3, T_4 and further generations some of the variants had certain characters resembling those of *N. silvestris*, one of the ancestors of *N. tabacum*. The most typical variants were nos. 801/1/25 *p* 18 and 801/20/20 *p* 12. Both had a similar appearance in respect to the habit of growth, and shape and colour of the flowers and leaves. Flower tubes in both ended gradually in a funnel-like opening, the flowers being pale pink. The flowers of variant 801/20/20/12 *p* 34 (cf. Pl. XVIII, fig. 8), is very much like the flowers of these two variants. In both $2n=48$. Plant 801/1/15 *p* 18 was partially fertile (× ×), while 801/20/20 *p* 12 was highly fertile (× × ×). The progenies of these two plants were relatively uniform. Segregation was noticed in leaf basis and in flower colour. A few T_4 offspring had almost ivory flower colour. Marked segregation was found in fertility (cf. Table VI). Four T_4 plants obtained by self-pollination of 801/1/25/18 had the following somatic chromosome numbers: 49, 50 and 48 (twice.) The offspring (T_5) of the plant 801/1/25/18 *p* 10 having 48 somatic chromosomes were very uniform morphologically.

Plants obtained in crossing variant 801/1/25/18 *p* 10 with *N. tabacum* var. *macrophylla* were not uniform in leaf basis (cf. Table IX). Flower colour of all these plants was lighter than in *macrophylla*, i.e. diluted red (dark pink).

T_4, variant 801/1/25/18 *p* 44 (Pl. XVIII, fig. 10), had light green leaves and very pale (almost ivory) pink flowers. It had $2n=48$. Slight irregularities (lagging chromosomes on the spindles) in the meiosis were noticed. The plant was partially fertile when self-pollinated (× ×) and fully fertile when pollinated with *N. tabacum macrophylla*. Most of the T_5 plants produced by self-pollination had almost ivory (pale pink) flower colour.

The hybrids grown from the cross-variant 801/1/25/18 *p* 44 × *N. tabacum* var. *macrophylla* were not uniform. Two plants had petiolate leaves with small wings on the petioles, and one plant had pink flowers (cf. Table IX).

In the next generation of variant 801/20/20 *p* 12 three plants were studied cytologically, one having 49 and two 48 somatic chromosomes. Plant 801/20/20/12 *p* 34 had $2n=48$ (Pl. XVIII, fig. 8).

Variant 801/20/20/3 *p* 7 (Pl. XVIII, fig. 7) also had characters resembling those of *N. silvestris*. The family in which this plant appeared was very much like the families grown from variants 801/1/25/18 and 801/20/20/12. The chromosome number of 801/20/20/3 was not studied, but its progeny from self-pollination varied very greatly (unlike 801/1/25/18 and 801/20/20/12 families) (cf. Table VI), differing in leaf basis, flower shape

and colour. Three of them had 47, 48 and 49 chromosomes. Variant 801/20/20/3 *p* 7, having 49 chromosomes, had very vigorous growth, large sessile leaves and very large pink flowers. It was partially fertile after self-pollination.

Among the progeny of plant 801/1/26 one plant was found (801/1/26 *p* 22) which was very much like *Nicotiana tabacum* var. *fruticosa* in habit of growth. It had a shorter vegetation period than var. *macrophylla* and the majority of its sister plants. Its leaves had long petioles, the wings on the leaf basis being completely reduced. Flower tubes were very narrow, ending gradually in a funnel, the ends of the petals forming very small angles, corolla colour red without the light star. (The star is characteristic for *N. tabacum macrophylla* (cf. Pl. XVIII, fig. 1) and it is not present in *N. tabacum fruticosa*.) It had 49 somatic chromosomes, one of them being exceedingly small (fragment-like). It had 92% viable pollen, and the position of its anthers above the stigma was such as to secure a good self-pollination; nevertheless, it was highly sterile (×), setting a very small amount of seeds after self-pollination. Its progenies were relatively uniform in habit of growth, flower shape, colour and leaf shapes. Segregation occurred in the presence or absence of small wings on the relatively long petioles. Out of twenty-seven, twenty had very small wings on the petioles, four had relatively small ones, and three had no wings. The plants also showed various degrees of fertility. Five plants were partially fertile, sixteen had very low fertility and six were completely sterile. Two of the plants studied cytologically had 48 somatic chromosomes, and two others had 48 + 1 very small one. It is interesting to note that the family 801/1/26/22 was more resistant to mosaic disease than the other families of the variants studied, and than the original *macrophylla* variety.

We may here call attention to the cytogenetic behaviour of two variants, viz. 801/1/26/22 *p* 16 and 801/1/26/22 *p* 6. 801/1/26/22 *p* 16 had traces of anthocyanine on the petioles. The flowers of the whole family were uniform (cf. Pl. XVIII, fig. 4). This variant had 49 somatic chromosomes, one of them being a very small one with median constriction. It usually formed 24 bivalents during the first meiosis. The small chromosome appeared usually as univalent. There were also P.M.C. in which the small chromosome was not noticed. In these cases it probably has conjugated with some of the normal bivalents, but the trivalent groups were difficult to distinguish because of the very small size of the "small chromosome". Variant 801/1/26/22 *p* 16 had very low fertility, when self-pollinated setting less than fifty seeds per capsule. The capsules were very small

and shrunken. In pollinating it with *N. tabacum* var. *macrophylla*, it formed large capsules like those of *macrophylla*, and set about as many seeds per capsule as the original variety. The type of the variant 801/1/26/22 *p* 16 was preserved in the subsequent generation. The plants of the family grown from seeds obtained by self-pollination had petiolated leaves with very small wings and flowers with narrow tubes. Three out of eight T_5 plants were highly fertile (cf. Tables VI and IX). One had 48 somatic chromosomes and two had 48+1 small chromosome.

F_1 from 801/1/26/22 *p* 16 × *N. tabacum* var. *macrophylla* had petiolated leaves like the variant. It should be pointed out that *petiolated* leaf basis usually behaves as a recessive character, though it may sometimes show intermediate inheritance when various strains of *N. tabacum* are crossed. It is very probable that we have here a case of duplication of a segment that includes the gene for petiolate leaf basis.

Two of these hybrids were studied cytologically; one had 48, the other had 49 chromosomes, the additional chromosome being the small one.

Variant 801/1/26/22 *p* 6 was a typical representative of tobacco plants with petiolate leaves having long petioles. The flowers were red and had narrow tubes, both being typical for the whole family. It had 48 somatic chromosomes. During the meiosis laggards as well as chromatin bridges were occasionally found (Pl. XIX, fig. 17). In studying the meiosis of this plant we found in two cases P.M.C. with enormously increased chromosome numbers. Univalents, bivalents, trivalents and quadrivalents were found in these cells. Such a cell, having 68 chromatin units, is given on Pl. XIX, fig. 18.

This variant had very low fertility when self-pollinated but quite normal (× × × ×) when crossed with the original form.

Variant 801/1/26/22, having a small additional chromosome, served for a special kind of investigation, in consequence of which we grew later on a further T_4 family of it from seeds obtained by self-pollination. To avoid confusion we shall designate this family as T_{4m}, and all the plants and their further progenies will have an "*m*" in addition to their numbers.

Twelve plants of this T_{4m} family, studied cytologically, gave the following results:

Variant 801/1/26/22 *p* 1*m* had 48 somatic chromosomes.

Variant 801/1/26/22 *p* 2*m* had 48+1 small=49 chromosomes.

Variant 801/1/26/22 *p* 3*m* had 48 chromosomes.

Variant 801/1/26/22 *p* 4*m* had 48+2 small=50 chromosomes.

Variant 801/1/26/22 *p* 5*m* had 48 chromosomes.
Variant 801/1/26/23 *p* 6*m* had 48 chromosomes.
Variant 801/1/26/22 *p* 7*m* had 48+1 small=49 chromosomes.
Variant 801/1/26/22 *p* 8*m* had 48+1 small=49 chromosomes.
Variant 801/1/26/22 *p* 9*m* had 48 chromosomes.
Variant 801/1/26/22 *p* 10*m* had 48 chromosomes.
Variant 801/1/26/22 *p* 11*m* had 48+1 small=49 chromosomes.
Variant 801/1/26/22 *p* 13*m* had 48+3 small=51 chromosomes.

These data show that from the variant 801/1/26/22 plants, with 0, 1, 2 and 3 small chromosomes appeared in the subsequent generation. The occurrence of three small chromosomes in variant 801/1/26/22 *p* 12*m* supplied evidence that the small chromosomes can be also transmitted through the pollen tubes. The small chromosome of T_{4m} plants (*p* 2*m*, *p* 7*m*, *p* 8*m*) usually appeared as a univalent during meiosis, but occasionally it was attached to a bivalent, forming a heteromorphic trivalent group in about 3% of the P.M.C. studied. In a few instances (*ca.* 0·8%) the small chromosome conjugated with a normal one, forming a heteromorphic bivalent, while the other normal chromosome appeared as a univalent.

Meiosis in variant 801/1/26/22 *p* 4*m*, having two additional small chromosomes, was more regular. It formed 25 bivalents in about 48% of the P.M.C., 24 bivalents and 2 univalents (the univalent chromosomes being the small ones) in *ca.* 47% of the P.M.C., and in about 5% of the P.M.C. the following cases were found: (1) a heteromorphic quadrivalent consisting of two normal and two short chromosomes, (2) one heteromorphic trivalent consisting of two normal and one small chromosome, and (3) a heteromorphic bivalent consisting of a normal and a small chromosome and two univalents, one being normal and the other one small. Variant 801/1/26/22 *p* 12*m*, having $2n=48+3$ small, formed less multivalent heteromorphic chromosome groups during the meiosis (*ca.* 1·2%) than *p* 4*m*. Small chromosomes appeared in the form of one bivalent and one univalent in about 58% of the P.M.C., in the form of three univalents in about 38% of the P.M.C. and as a homomorphic trivalent in *ca.* 2·8% of P.M.C.

In order to study the transmission of the small chromosome through the egg cells and through the pollen, variant 801/1/26/22 *p* 2*m*, having $2n=48+1$ small, was crossed in both directions with *N. tabacum* var. *macrophylla*. The presence of the small chromosome was studied in the meiosis of F_1 generation on aceto-carmine preparations. Fifteen F_1 plants of the cross *p* 2*m* × *macrophylla* out of eighteen cytologically

studied had the small chromosome. In two plants the small chromosome was not present. One plant had even two small chromosomes which indicated that egg cells (1) with no small chromosomes, (2) with one small chromosome, and (3) with two small chromosomes, were viable. The latter probably originated after division of the small chromosome during the first meiosis and inclusion of both halves into one anaphasal group during the second meiotic division; the latter anaphase giving rise to an egg cell with two small chromosomes.

Three F_1 plants of the cross *macrophylla* × *p* $2m$, out of twenty-one studied cytologically, had one small chromosome, while the other eighteen plants had no small chromosome. These data show that the small additional chromosome can be more easily transmitted through the egg cell than through the pollen. It seems that pollen tubes having the additional small chromosome grow much slower than those having 24 chromosomes. The transmission of two small chromosomes gives rise to homozygous plants with $2n=50$ in respect to the small chromosomes.

The behaviour of the small chromosome during the meiosis of the hybrids with *macrophylla* was similar to that in the variants with one and two small chromosomes.

It was mentioned above that variant 801/1/26/22 and its progeny were very similar to *N. tabacum* var. *fruticosa*, and it was of interest to enquire into the possibility of increasing the fertility of this strain to produce new variants that might compete with the original form. Their short vegetation period marked them out as favourable material.

We found that T_4 plant 801/1/26/22 *p* $9m$ set the largest amount of seeds per capsule (700–1000). By further selection of the most fertile T_{5m} variant we obtained in T_{6m} plants with about 1000–1200 seeds per capsule on self-pollination. We are now growing a T_{7m} generation, and the fertility of this *fruticosa*-like variant, with $2n=48$, suggests that it will compete favourably with the original form. Indeed, such variants with shortened vegetation period as in our case may well be better suited to new areas with shorter summers.

We also grew on variant 801/1/26/22 *p* $4m$ in which $2n=50$, i.e. a variant with 25 pairs (the small chromosome being in homozygous condition), until T_{7m}, and on selecting in each generation variants with $2n=50$, we found in T_{5m} about 63 % with $2n=50$, and in T_{6m} about 75 %. In T_{7m} we studied only twenty-four plants, of which twenty-one were homozygous for the small chromosome, i.e. about 88 %. These observations also showed that in some variants the relative stability of

the new karyotypes increases with the increase of the number of generations.

Alkaloid content of the temperature variants

Biochemical analysis (quantitative and qualitative) carried out by a series of investigators, especially those by Shmuck and his coworkers upon the alkaloid content in various species of the genus *Nicotiana* and particularly in *N. tabacum*, showed that the latter species contains chiefly the alkaloid nicotine, though accompanied by traces of some other alkaloids. The characteristic property of nicotine is that it can be easily distilled (or rather sublimated) with water vapour, while the other alkaloids remain behind. Nicotine reacts with picric acid, forming nicotine picrates, yellowish (pale lemon colour) needle-like crystals with M.P. 218° C.

By Keller's method one determines the total amount of alkaloids, while by Bertrand's method one determines the distillating alkaloid, which is chiefly nicotine. The difference represents the non-distilling (non-sublimating) alkaloids other than nicotine. In *N. tabacum* these differences were very small (Tables X and XI), whereas in *N. silvestris* (one of the ancestors of *N. tabacum*) they are considerable. Hence *N. silvestris*, according to Shmuck, contains a large percentage of normicotine.

Table X gives the alkaloid contents of *N. tabacum macrophylla* (control) and of some T_3 temperature variants grown in the same conditions. In Table XI the alkaloid content of *macrophylla* (control) and of some T_4 temperature variants is given.

The alkaloid content of *N. tabacum macrophylla* grown as a control of the variants of T_3 and T_4 shows little variation. It had 0·98 % alkaloid, of which 0·94 % was nicotine, and in the next generation it had 0·94 % alkaloid, 0·91 % being nicotine. Variants of T_3 and T_4 had alkaloid and nicotine contents that differed greatly from those of the original *macrophylla* plant. T_3 variants 801/1/24/28 and 801/8/23/11 had the lowest alkaloid contents (Table X), namely 0·65 %, while plant 801/1/25/18 had 2·00 % alkaloids. The percentage of the nicotine content as compared with the other alkaloids also differed very greatly. The nicotine content of T_3 variant 801/1/24/28, for example, was only 30·7 % of the whole alkaloid content, while the nicotine content of T_3 variant 801/2/21/5 was 93·9 % of the whole alkaloid content, i.e. approximately as high as in *macrophylla*.

Some T_4 variants differed from the original form in respect of the

TABLE X

Alkaloid content in some variants of T_3 generation grown at equal conditions

No.	Plants	Somatic chromosome nos.	Total amount of alkaloids determined by Keller's method % in respect to the dry substances	Nicotine content determined by Bertrand's method		Other alkaloids than nicotine (differences)	
				% in respect to the dry substances	% in respect to the total amount of alkaloids	% in respect to the dry substances	% in respect to the total amount of alkaloids
1	*N. tabacum* var. *macrophylla*	48	0·98	0·94	95·9	0·04	4·1
2	801/1/20 *p* 1	48	1·50	1·37	91·3	0·13	8·7
3	801/1/20 *p* 21	47	1·34	1·11	82·1	0·23	17·0
4	801/1/24 *p* 28	49	0·65	0·20	30·7	0·45	69·3
5	801/1/25 *p* 18	48	2·00	1·85	92·5	0·15	7·5
6	801/1/26 *p* 18	47	0·80	0·78	87·5	0·02	12·5
7	801/1/26 *p* 22	48 + 1 fr.	1·19	1·06	89·1	0·13	10·9
8	801/2/21 *p* 5	48	1·31	1·23	93·9	0·08	6·1
9	801/3/23 *p* 2	48	1·01	0·83	82·2	0·18	17·8
10	801/8/23 *p* 11	50	0·65	0·46	70·8	0·19	29·2
11	801/20/20 *p* 3	49	1·51	1·38	91·4	0·13	8·6

alkaloid and nicotine contents in a similar way to those of the T_3 generation (cf. Table XI).

T_4 variants 801/1/24/28/14, 801/1/24/28/15 and 801/20/20/3/14, all chromosome aberrants, had the smallest percentage of alkaloids (0·324, 0·291 and 0·486 respectively), and the smallest percentage of nicotine in respect to the total alkaloid contents (17·9, 59·1 and 10·3 respectively). M.P. of the alkaloid picrates of 801/1/24/28/14 and 801/20/20/3/14 variants was 178–180° C., while in all the other T_4 variants (except plant 801/1/24/28/15) they were 218, 218–219, and 217–218° C., though somewhat lower in 801/8/23/11/17 ($2n=51$), viz. 212° C. Variant 801/1/24/20/15 was the most outstanding in this respect, since its alkaloid picrates melted at three different gradually increasing degrees, namely, 190–220–260.

These data show that the hereditary changes induced by abnormal temperatures condition both quantitative and qualitative changes in the biochemistry of the plant, as well as morphological ones.

Discussion and conclusions

The problem of the induction of hereditary variations by extreme temperatures (*ET*) may be conveniently divided into three parts, viz. (1) the mode of action of the *ET* and the kind of the primary hereditary changes induced, (2) production of secondary hereditary changes and the transmission of the primary and secondary hereditary changes through the subsequent generations, and (3) the survival value of the hereditary variations (primary and secondary).

The first point may be subdivided into (*a*) hereditary changes and their sequences induced in the soma, and (*b*) hereditary changes and their sequence induced during gametogenesis.

Hereditary variations induced by *ET* seem to result from (1) certain biophysical states, conditioned by *ET*, and (2) certain deviations in the procedure of the biochemical processes directed by the *ET*. It is unfortunate that we know very little about the biophysical state of the cell elements at various temperatures, or about the deviations from the process of biochemical reactions in living cells at various temperatures. We may, however, consider briefly what is known and attempt to interpret some of the results obtained in our experiments.

Cytoplasm and nuclear elements of the living cells consist chiefly of protein colloids, the viscosity of which is influenced by the temperature. Some temperatures increase the viscosity of the cytoplasm, others decrease it.

TABLE XI

Alkaloid contents of some temperature variants of F_4 generation grown at equal conditions

No.	Plants	Somatic chromosome nos.	Total amount of alkaloids determined by Keller's method % in respect to the dry substances	Nicotine content determined by Bertrand's method		Other alkaloids than nicotine (differences)		Melting points of the alkaloid picrates $T°$ C.
				% in respect to the dry substances	% in respect to the total amount of alkaloids	% in respect to the dry substances	% in respect to the total amount of alkaloids	
1	*N. tabacum* var. *macrophylla*	48	0·94	0·91	96·8	0·03	3·2	218
2	801/1/20/1 *p* 10	48	1·782	1·618	90·8	0·164	9·2	217–218
3	801/1/24/28 *p* 14	49	0·324	0·055	17·9	0·269	82·1	178–186
4	801/1/24/28 *p* 15	47	0·291	0·172	59·1	0·119	40·9	190–220–260
5	801/1/25/18 *p* 27	50	1·636	1·155	70·7	0·478	29·3	218
6	801/1/26/18 *p* 27	47	1·20	0·86	71·7	0·34	28·3	218
7	801/1/26/22 *p* 6	48	0·502	0·447	89·1	0·055	10·9	219
8	801/1/26/22 *p* 16	48 + 1 fr.	0·955	0·904	94·7	0·051	5·3	217–218
9	801/1/26/22 *p* 17	49	1·117	1·080	96·6	0·037	3·4	218
10	801/3/23/2 *p* 10	48	0·955	0·924	96·7	0·031	3·7	218
11	801/3/23/2 *p* 25	49	1·247	1·235	99·0	0·012	1·0	218
12	801/3/23/2 *p* 28	48	0·826	0·778	94·2	0·048	5·8	215–217
13	801/8/23/11 *p* 16	50	0·955	0·678	71·0	0·277	29·0	218
14	801/8/23/11 *p* 17	51	0·816	0·774	93·0	0·042	8·4	212
15	801/20/20/12 *p* 2	47	0·906	0·941	94·5	0·095	5·5	218–219
16	801/20/20/12 *p* 3	48	0·761	0·739	97·1	0·022	2·9	218
17	801/20/20/3 *p* 14	50	0·486	0·055	10·3	0·431	88·7	178–180
18	801/20/20/3 *p* 30	48	1·101	0·976	88·6	0·125	11·4	218

In studying the changes of the cytoplasmic viscosity in *Comungia*, Heibrunn (1924, 1928) plotted a W-like curve with higher points (higher viscosity) −1, 15 and 32° C. and with lower points (lower viscosity), 3 and 31° C. Němec (1901) found that in plants an increase in the cytoplasmic viscosity takes place at 6° C.

High temperatures lethal to living cells induce an irreversible coagulation of the cytoplasm. These temperatures induce first an increase of the viscosity and then coagulation. Temperatures somewhat below lethal induce an increase in cytoplasmic viscosity. Temperatures somewhat below sublethal (but still high) lead to a decrease in cytoplasmic viscosity.

An abnormal increase in cytoplasmic viscosity, whether due to high or low temperature, has a marked influence on chromosomal aberrations. Increased viscosity through low temperature diminishes chiasma frequency and even the number of bivalents in species hybrids (cf. Kostoff, 1930*b*, and unpublished). The same effect has been found in a few cases when the plant is exposed to extremely high (sublethal) temperature. Higher viscosity also tends to prevent chromosome conjugation during leptotene. Homologous chromosomes would move less effectively toward one another in more viscous media (when the attraction forces are the same). This leads to an increase of the chiasma frequency in less viscous and to a decrease in more viscous cytoplasm. In the latter case univalent chromosomes may appear during the first meiotic division, and their random distribution during the first meiosis leads to the formation of gametes with abnormal chromosome numbers ($n \pm a$). Since chiasmata represent crossing-overs (cf. Darlington, 1937), and since temperatures, inducing changes in cytoplasmic viscosity also cause changes in chiasma frequency, it is clear that temperatures would influence the crossing-over values. Such statements have been made long ago (Plough, 1917, 1921; Plough & Ives, 1932; Stern, 1926).

Increased protoplasmic viscosity leads also to somatic chromosome doubling. The chromosomes (or rather their centromeres) divide during the metaphase, but in a too viscous medium they cannot reach the poles and form a "tetraploid" nucleus on the equator or a binucleate cell which, during the subsequent division, can give rise to two tetraploid cells when the spindles of both metaphasal plates fuse (nuclei of syncytia divide synchronously). Randolph's method (1932) of chromosome duplication under the influence of high temperatures rests on this principle. Increased cytoplasmic viscosity induced by abnormal temperatures leads occasionally to meiotic chromosome doublings (Kostoff, 1931 *c*, *d*).

Another category of hereditary change induced by *ET* are chromosome dislocations. Peto's (1935) and Shkvarnikov's (1936) observations as well as our own showed that *ET* leads to exchange of parts in somatic cells. Direct observations, as well as the new types of chromosomes found in somatic cells after treatment, show definitely that exchange of parts between non-homologous chromosomes (and obviously between homologous ones also) takes place.

The next questions that arise are: (1) What types of chromosome rearrangements take place? and (2) What is the "mechanism" that regulates this phenomenon?

Direct observation upon the mitotic metaphases in material fixed immediately after treatment showed that exchange of parts between morphologically dissimilar chromosomes takes place. We had the impression that this phenomenon, like simple crossing-overs between chromosomes with inversions (in inverted segments), conditions the formation of exceedingly small chromosomes (*versus* long).

In studying meiosis in T_0, T_1 and the subsequent generations, we found abnormalities suggesting the following types of chromosome rearrangements.

(1) *Translocations, duplications.* The appearance of quadrivalents in variants with $2n=48$ and in trisomics with $2n=49$ when in the latter a quadrivalent and a univalent were found could be interpreted by postulating translocations. The formation of ring trivalents in a series of variants found during diakinesis also suggested translocations. Some of the latter might be primary of the type AB–BC–CA, others secondary of the type AxB–BxB–BxA, (AB–BB–BA).

(2) *Inversions, fragmentations.* The occurrence of chromatin bridges during the meiotic anaphases (some of which persisted even during the second metaphases) could be interpreted as bicentric chromatids resulting from crossing-over in inverted regions. The discovery of small fragments during diakinesis in variants forming chromatin bridges suggested a precocious separation of the fragments resulting from the activity of chromosome repulsions during diakinesis. Breakage (or perhaps the tearing of very viscous colloids such as the chromosomes) of the bicentric chromatids (chromatin bridges) also seems to lead to the formation of small chromosomes.

(3) *Deletions, deficiencies, "duplication-deficiencies".* Heteromorphic pairs found quite often during meiosis could be interpreted by postulating

deletions, deficiencies, and duplications. Exchanges of parts between non-homologous chromosomes in the soma may ultimately lead to "duplication" or "deficiency". Heteromorphic bivalents (one chromosome much longer than the other) with two terminal chiasmata found during diakinesis strongly suggested that the shorter one might have undergone deletion. Direct evidence for this was supplied in studying the chromomeres during the diplotene, when one chromosome of a heteromorphic pair has many more chromomeres between two terminal or subterminal chiasmata than the other. Deficiencies were also detected in a similar way. Chromosome pairs were found during diplotene in which one chromomere of a chromosome was lacking. We had here a loss but not a gain, since during the meiosis 24 pairs but no multivalents were found. On the contrary univalents were occasionally observed.

(4) *Numerical changes.* Temperature variants with $2n=47$, 48, 49, 50 and 51 chromosomes were obtained, some of them being definitely altered.

In the works by Jollos (1933, 1934), Peto (1937) and others, gene mutations were stated to have occurred. The segregations that we observed in some F_2 generations grown from crosses between *N. tabacum* var. *macrophylla* and some temperature variants with 48 chromosomes, strongly suggested that some new characters observed in the temperature variants might be due to gene mutations. Considering, however, the fact that *N. tabacum* is an allopolyploid in which various kinds of deficiencies and deletions might be viable, and that hereditary changes due to certain chromosome alterations might behave like gene mutations, we cannot positively affirm that we are concerned with gene mutations. For it seems possible that some of the gene mutations recorded by other investigators might have been due either to losses or to duplications. And here we may stress the difficulties in the genetic analysis of the hereditary changes induced in our material owing to (1) chromosome alterations that might mask gene mutations, and (2) selective fertilization due to differences in the rate of the pollen-tube growth of gametes having various changes, chromosomal or genic. The data given in Tables III–XI, as well as the cytological results, serve to emphasize these difficulties.

In order to estimate correctly the additional complications in the primary hereditary alteration induced by temperature, the possibility of secondary hereditary alterations due to conjugations and crossing-over between partially homologous chromosomes newly changed and unchanged must be considered.

This problem, as well as the evolutionary significance of the tem-

perature variants, can be fruitfully discussed in the light of researches upon the problem of the origin of *N. tabacum* species. The theoretical arguments advanced by Clausen (1927) about the origin of *N. tabacum* were: The hybrids *N. tabacum* ($n=24$) × *N. silvestris* ($n=12$) and *N. tabacum* × *N. tomentosa* ($n=12$) formed 12 bivalents and 12 univalents, while haploid *N. tabacum* and the F_1 *N. silvestris* × *N. tomentosa* usually formed 24 univalents. On the basis of these arguments he assumed that *N. tabacum* has probably originated by chromosome doubling in F_1 *N. silvestris* × *N. tomentosa*. Kostoff (1930*b*, 1931*d*, 1933*a*, 1934, 1936*b*, 1938 *b*, *c*) tested this theory by crossing *N. tabacum* var. *macrophylla* with pollen of F_1 *N. silvestris* × *N. tomentosiformis*, produced at relatively high temperature (1931*d*). Most of the triple hybrids so formed had whole genoms of *N. tabacum* (24), *N. silvestris* (12) and *N. tomentosiformis* (12). They had relatively normal meiosis, since *silvestris* chromosomes conjugated with 12 *N. tabacum* chromosomes, while *tomentosiformis* chromosomes conjugated with the other 12 *tabacum* chromosomes. Kostoff used *tomentosiformis*, instead of *tomentosa*, because the F_1 *N. silvestris* × *tomentosiformis* hybrids were more *tabacum*-like (1933*a*). *N. tomentosa* is very closely related to *N. tomentosiformis*; their hybrids usually formed 12 bivalents, had normal meiosis, and were fully fertile. Further evidence as to the origin of *N. tabacum* (Kostoff, 1938 *b*, *c*) was the production of the fertile *N. tabacum*-like allotetraploid hybrid *N. silvestris-tomentosiformis* by gradual accumulation of genoms, which gave fertile hybrids with almost normal meiosis when crossed with *N. tabacum* varieties (Kostoff, unpublished).

Since the additional complications in the heritable variations primarily produced by temperature would be chiefly conditioned by cross-overs between *silvestris* and *tomentosiformis* chromosomes as well as between the reorganized and non-reorganized chromosomes, we must consider here the degree of homology between *silvestris* and *tomentosiformis* chromosomes in the F_1 hybrid *silvestris* × *tomentosiformis*, of which the meiosis should be (and really is) very much like that of the haploid *N. tabacum*. Text-fig. 6 shows the frequency of the bivalents in F_1 *silvestris* × *tomentosiformis* hybrids, which usually showed one chiasma during the metaphase. About 61·2% of the P.M.C. had at least one bivalent with one chiasma. Two bivalents or more than two (up to 5) occurred rarely. The same chromosome behaviour was found in the haploid *N. tabacum* (Kostoff, unpublished; cf. Clausen & Mann, 1924; Lammerts, 1934, etc.).

These observations showed that in the monosomic, trisomic, and

polysomic variants, "*silvestris*-type" univalent chromosomes can occasionally conjugate with "*tomentosiformis* type", cross-over and give rise to reorganized chromosomes in the way that partially homologous chromosomes behave in species hybrids (cf. Kostoff, 1935*d*, 1937*a*, 1938*d*). The reorganized chromosome produced under the influence of abnormal temperatures in the variants studied may behave in a similar way.

In connexion with the primary, and especially with the secondary chromosome alterations occurring in hybrids produced by crossing two primary variants (such as our T_2 variants and their progenies) the following question arises, viz. Is some degree of partial homology necessary for chromosome conjugation and crossing-over, or might these processes sometimes take place between the heterochromatic, genetically inert regions of non-homologous chromosomes? If conjugation and crossing-over occasionally take place in the heterochromatic regions of non-homologous chromosomes during meiosis, or even in the somatic cells, the secondary chromosome rearrangement might be very significant in allopolyploid plants like *N. tabacum*.

Cytological studies during the last few years upon chromosome conjugations in heterochromatic regions shows that this process occurs in certain material, e.g. in the salivary glands of *Drosophila* and other Diptera, in *Drosera* tentacles, in some plant galls, and probably in some species hybrids as well as under the influence of certain external agents (temperature, X-rays, etc.) (for literature see Kostoff, 1938 *a*, *e*). Conjugation of the heterochromatic regions in a common chromocentre in *Drosophila* is the best example in this respect, but a series of good examples in the plant kingdom can be also recalled (cf. Kostoff, 1938 *a*, *e*).

Primary as well as secondary chromosome rearrangements were responsible for the numerous numerical and structural variants that we described in this paper. The characters involved were: (*a*) morphological, (*b*) physiological, and (*c*) biochemical. The morphological characters involved colour as well as shapes and quantities. There were giants and dwarfs with various habits of growth, with normal and deformed leaves and flowers. Variants with different expressions of the leaf petioles and the wings on the petioles, corrugations, different angles of the apex, different leaf indexes (length : breadth), etc., were raised. Temperature variants with quite different numbers of leaves were grown. Variants with different flower sizes and shapes, with different lengths of the stigmas and anthers, etc., were also raised. The colour of the leaves and flowers differed in many variants. There were variants with deep green leaves and others with lighter ones. The gradation of flower colour from

deep red to pale pink (almost ivory) were the most striking characters. Morphological as well as the physiological characters (as, for example, the changes in the vegetation period and the changes in resistance) are of great evolutionary significance. The changes in the contents of alkaloids, qualitatively and quantitatively, are also of significance. Similar changes in the alkaloid content were also determined by Popoff, Kostoff & Kendall (1931) in *N. tabacum* variants obtained by Kostoff & Kendall (1931) by wounding the anthers before or during meiosis. Since a large number of the hereditary changes of the temperature variants were probably due to "duplications" v. "deficiencies" (also deletions), very many of the variants had *silvestris*-like and *tomentosiformis*-like characters.

Hence many of these variants, although called "temperature variants", were merely derived from the primary variants obtained directly under the influence of extreme temperatures. The "indirect" changes, which were chiefly due to fresh numerical changes and to secondary chromosome rearrangements following crossing-over between partially homologous[1] chromosomes, to subsequent recombinations and ultimate homozygosis, were undoubtedly much more effective than those resulting directly from the temperature effect. Nevertheless, the latter conditioned the former.

We may here recall a similar case (Kostoff, 1931*b*), in which *Capsicum annuum* plants exposed to abnormal temperature presented abnormal meiosis. After selfing there appeared in T_1 one trisomic ($2n+1=25$) and one structural heterozygote ($2n=24$) which during meiosis formed 10 bivalents and 1 quadrivalent. In the trisomic $12^{II}+1^{I}$ or $11^{II}+1^{III}$ were found, and among its progeny were variants with numerical and structural changes (Kostoff, unpublished).

These studies show that small changes induced by abnormal temperatures in nature may further lead in some organisms to more effective changes, and here we may mention some observations of other authors in support of our suggestion. In the second generation of monosomic spelt wheats, Nishiyama (1928) obtained the expected types 20^{II}, $20^{II}+1^{I}$, and 21^{II} and unexpected types with $18^{II}+3^{I}$, $19^{II}+1^{I}$, $19^{II}+2^{I}$ and $19^{II}+3^{I}$. Some of these might result from structural changes following conjugation and crossing-over between the single chromosome and some of those presented in pairs. A more convincing case is the plant

[1] Probably also between the heterochromatic regions of the non-homologous chromosomes.

with a small chromosome (fragment) obtained by Nishiyama (1933) on selfing a monosomic oat (*Avena*).

Plants with structural changes were also obtained on selfing monosomics of *Nicotiana tabacum* (Clausen, 1931, 1932; Olmo, 1936). Lammerts (1932) also observed variants in the progeny of *N. rustica* monosomics. The complexity of the segregations often obtained from monosomic and trisomic "speltoids" from *vulgare* wheats reported by many authors (Håkansson, 1932, and others) cannot be merely interpreted by the expected numerical changes without postulating structural alterations. "Hexaploid" wheats and oats as well as *N. rustica* are allopolyploid species like *N. tabacum*.

Large numbers of forms that arise from allopolyploids when the F_1 hybrids, from which they originate, show partial allosyndesis during meiosis were fully discussed by Kostoff (1938*d*) in connexion with the progenies of *Nicotiana glauca-Langsdorffii* allopolyploids. Allopolyploids *N. silvestris-tomentosiformis*, experimentally obtained (Kostoff, 1936*a*, 1938*b*, *c*), also "segregated", giving rise to some numerical as well as structural variants, since in some P.M.C. of the allopolyploids, univalents and polyvalents were found. In the light of this discussion the results obtained by Leliveld (1937) in studying some progenies by Kostoff's triple fertile hybrids *N. triplex* are to be expected, since *N. triplex* also formed occasionally multivalents (Kostoff, 1933*a*).

Our "temperature" variants clearly point to the origin of many of the varieties now known in *N. tabacum*. Non-conjunctions induced by external conditions (chiefly temperature) lead to numerical changes, while these give rise to structural hybrids. Occasional multivalency leads to the same results. Multivalency and univalency have, no doubt, occurred more frequently in the earliest formed *N. tabacum* plants (allopolyploids of *N. silvestris-tomentosiformis*). Hybridizations of the new variants have given rise to new combinations, and so the number of the varieties has been increased. We may recall here that numerical changes in *N. tabacum* (trisomic and monosomic) have occasionally been found in nature. Some *N. tabacum* varieties differ structurally. Some varietal hybrids, for example, with *N. tabacum* var. *calyzina* (and other varieties), represent structural hybrids (Kostoff, unpublished).

One of the most essential problems in experimental evolution and plant breeding is the survival of the new organisms arising in nature or experimentally produced. Our variants were usually less fertile when they first arose, but their fertility rapidly increased in a few generations, approaching in some of them that of the original form. New physiological

characters, e.g. shortening of the vegetation period, increased resistance, etc., favour the survival of the organism and its spread into new areas. Plants with shorter vegetation period might occupy areas more distant from the equator with shorter summers than those with long vegetation periods. Plants with increased numbers of leaves, with altered chemistry (quantitatively and qualitatively), etc., are important from an agricultural point of view.

All this goes to show that extreme temperatures are a powerful factor in the induction of hereditary variations, either directly or through the changes, further complications arising independently from them. Temperature is a factor to which all living beings are exposed in nature. It induces heritable as well as non-heritable variations and so affords abundant material for natural and artificial selection. Parasites are another important natural factor for the induction of hereditary variation. Viruses and bacteria, mites and gall wasps, etc., induce abnormalities in the process of cell division, meiosis and mitosis, and these may lead to the formation of heteroploid and polyploid sex and somatic cells (Kostoff, 1930*a*, 1933*b*, *c*, 1936*b*, 1938*f*; Kostoff & Kendall, 1929*a*, *b*, 1930 *a*, *b*, 1931, 1932, 1933, 1934; Kendall, 1930*a*, *b*, etc.). Parasites act upon the plant tissues by their *chemical excretions* and by the *wounds* which they cause. And here we may point out that the "decapitation" method for inducing polyploidy (to which may also add "heteroploidy"), recommended by Winkler (1916) and Jørgensen (1928) and widely practised during the last decade is based upon "wounds". (These seem to increase the cytoplasmic viscosity.) At the present time, however, the most effective methods for inducing polyploidy and heteroploidy are, no doubt, the *chemical* methods in which *colchicine* (Ludford, 1936; Blakeslee, Avery, *et al.*, 1937; Kostoff 1938*g*, *i*, *j*, *k*; Walker, 1938; Gavaudan, 1938; Margenot, 1938; Simonet & his coworkers, 1938; Levan, 1938; Györffy, 1938, etc.) and *acenaphthene* (Kostoff, 1938*g*, *h*, *i*, *j*, *k*, etc.) are applied. These two agents act chiefly by interfering with the formation of achromatic figures, but there are also chemicals which act by changing the cytoplasmic viscosity.

SUMMARY

Abnormal temperatures (*AT*) induce in *Nicotiana tabacum* var. *macrophylla* irregularities in mitosis and especially in meiosis which lead to numerical chromosome alterations, aneuploid and euploid. They also induce structural chromosome changes.

Among the progeny of treated plants structural and numerical variants of various kinds were found.

By crossing among themselves the extreme variants thus produced, and by further breeding, paying greater attention to the more extreme ones, we obtained a large number of new variants, which behaved quite differently.

Chiasma formations between normal and structurally changed chromosomes, between partially homologous unchanged, and probably also between non-homologous ones, conditioned secondary structural changes and variations.

Some of the variants are considered from an evolutionary point of view in connexion with the origin of the species *N. tabacum* and its varieties.

The roles of abnormal temperatures and of other factors that induce hereditary variations in nature are discussed.

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EXPLANATION OF PLATES XVIII–XIX

PLATE XVIII

Flowers of the original form and of nine experimentally produced extreme variants.

Fig. 1. *Nicotiana tabacum* var. *macrophylla*—the original form ($2n = 48$).

Fig. 2. Variant S01/7/23 *p* 3; $2n = 50$. (Sessile leaves.)

Fig. 3. Variant S01/10/24 *p* 2; $2n = 49$. (Sessile leaves.)

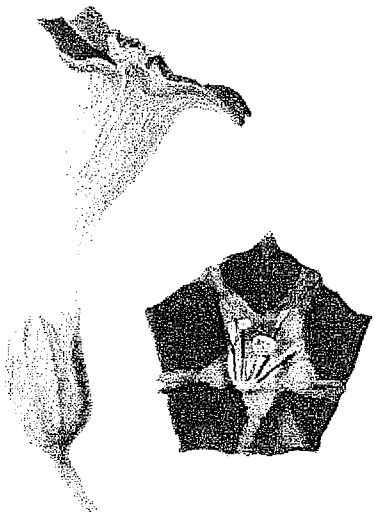
Fig. 4. Variant S01/1/26/22 *p* 16; $2n = 48 + 1$ small. (Petiolate leaves with small wings on the petioles.)

Fig. 5. Variant S01/1/25 *p* 5; $2n = 48$. (Elongated leaves with long petioles.)

Fig. 6. Variant 801/1/24/28 *p* 2; $2n=48+2$ small. (Intermediate basis of the leaves.)
Fig. 7. Variant 801/20/20/3 *p* 7; $2n=49$. (Sessile leaves.)
Fig. 8. Variant 801/20/20/12 *p* 34; $2n=48$. (Sessile leaves.)
Fig. 9. Monosomic variant 801/1/26/18 *p* 27; $2n=47$. (Sessile elongated leaves.)
Fig. 10. Variant 801/1/25/18 *p* 44; $2n=48$. (Sessile leaves.)

PLATE XIX

Fig. 1. Diakinesis in a P.M.C. of variant 801/1/21 *p* 3. Note one univalent and a trivalent ring.
Fig. 2. First meiotic metaphase (side view) of variant 801/20/20 *p* 2. Note univalent and trivalent.
Fig. 3. Diakinesis in variant 801/7/23 *p* 3. Note polyvalent and univalent chromosomes.
Fig. 4. Diakinesis in variant 801/7/23 *p* 3. Note small chromosomes (fragments).
Fig. 5. Metaphase (side view) of variant 801/7/23 *p* 3. Note a trivalent and two small chromosomes (fragments).
Fig. 6. First anaphase with a chromatin bridge. Variant 801/7/23 *p* 3.
Fig. 7. First anaphase with laggards. Variant 801/7/23 *p* 3.
Fig. 8. Second metaphase with 24 + 26 chromosomes. Variant 801/7/23 *p* 3.
Fig. 9. First anaphase with laggards and delayed separation of a probably heteromorphic bivalent. Variant 801/10/24 *p* 2.
Fig. 10. First metaphase (side view) with a trivalent (probably heteromorphic) and a univalent. Variant 801/1/24/28 *p* 14.
Fig. 11. Second metaphase with 25 : 26 chromosomes, suggesting a division of univalent chromosomes during the first meiosis (cf. text). Variant 801/1/24/28 *p* 14.
Fig. 12. First metaphase, side view, a small univalent on the spindle. Variant 801/1/24/28 *p* 17.
Fig. 13. First anaphase with lagging small (short) univalent; other univalents divide. Variant 801/1/24/28 *p* 17.
Fig. 14. First metaphase (side view) with a small univalent (outside of the plate), and a polyvalent chromosome. Variant 801/1/24/28/2 *p* 4.
Fig. 15. First anaphase, the small univalent chromosome divides on the spindle. Variant 801/1/24/28/2 *p* 4.
Fig. 16. Second anaphase with laggards, one being the fragment. Variant 801/1/24/28/2 *p* 4.
Fig. 17. Second metaphase each plate has 24 chromosomes. The plates (rather one chromosome of one plate with another from the other plate) are connected with chromatin bridge formed during the first meiosis and persisting until the second metaphase. Variant 801/1/26/22 *p* 6.
Fig. 18. First meiotic metaphase with about 68 units (univalents, bivalents and multivalents) from a pollen mother cell, probably with multiple chromosome number. Variant 801/1/26/22 *p* 6.



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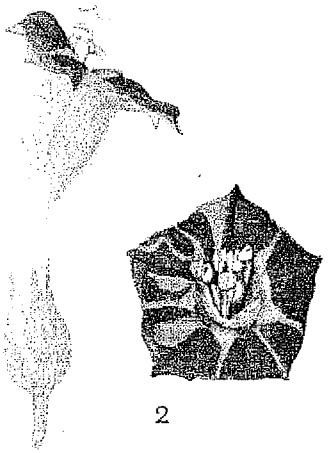

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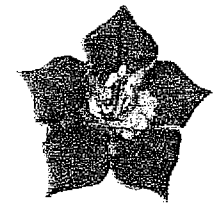

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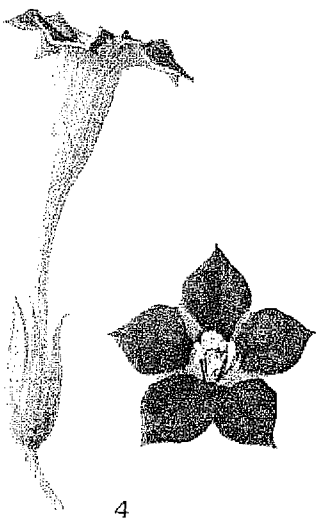

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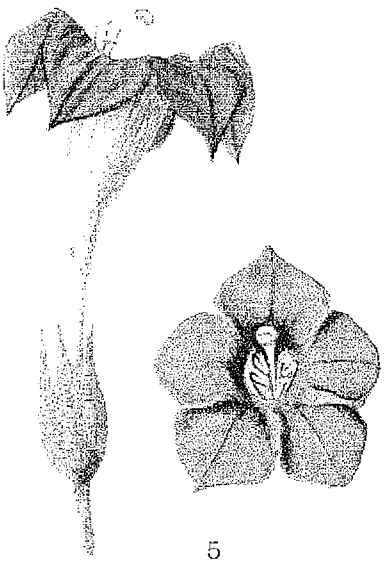

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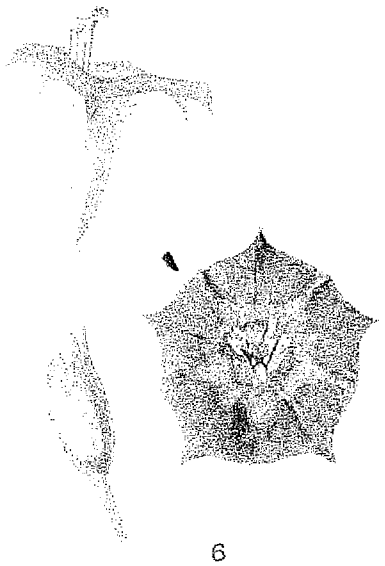

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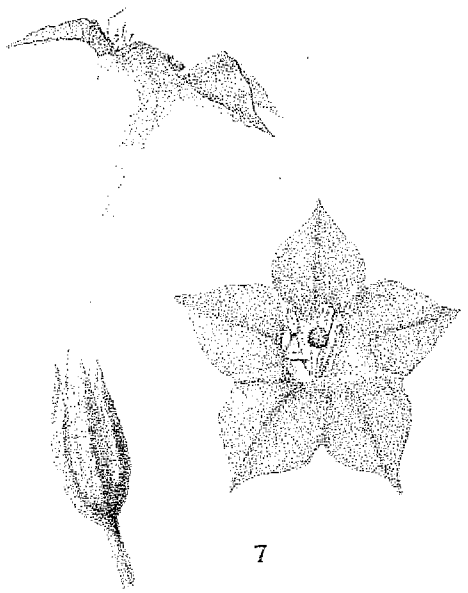

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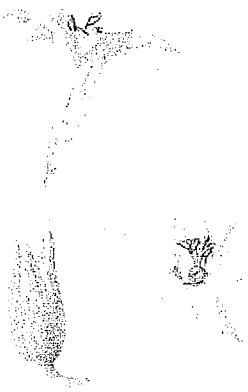

9

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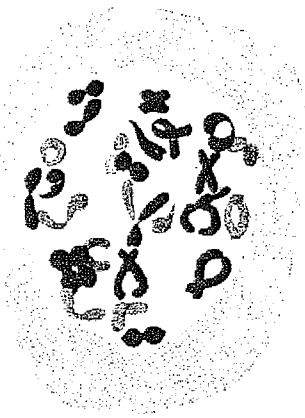
Fig. 1.

Fig. 2.



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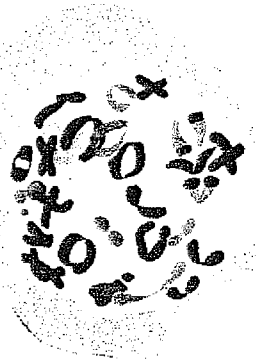
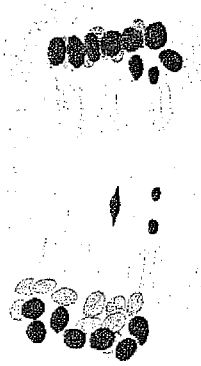


Fig. 4.

Fig. 5.

Fig. 6.



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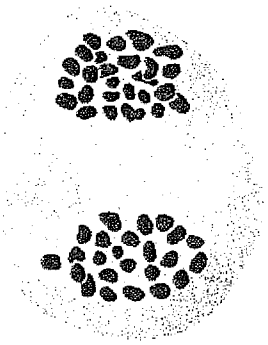

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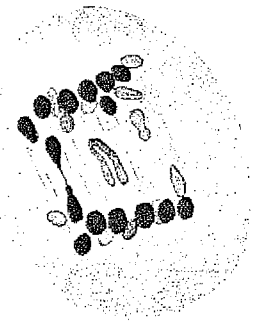

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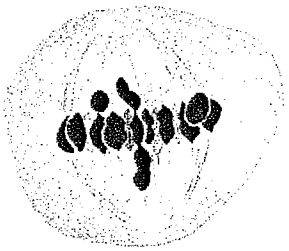


Fig. 10.

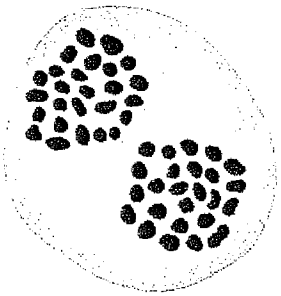


Fig. 11.

Fig. 12.

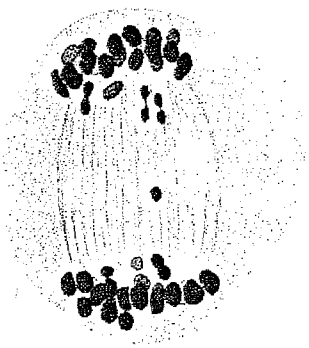
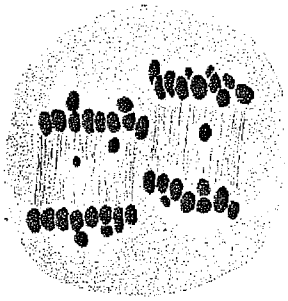


Fig. 13.

Fig. 14.

Fig. 15.



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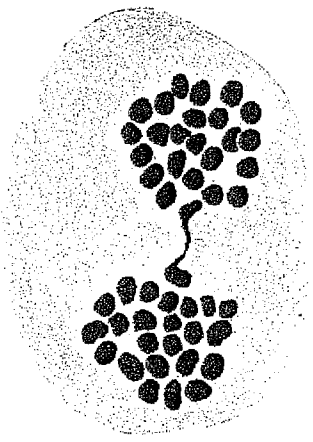

Fig. 17.

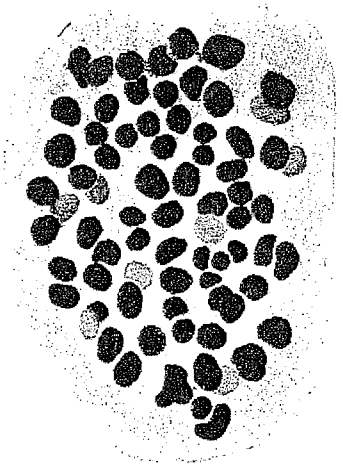

Fig. 18.