STUDIES ON POLYPLOID PLANTS

XXI. CYTOGENETIC BEHAVIOUR OF THE ALLOPOLYPLOID HYBRIDS *NICOTIANA GLAUCA* GRAH. × *NICOTIANA LANGSDORFFII* WEINM. AND THEIR EVOLUTIONARY SIGNIFICANCE

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(With Plate IV and Thirty-six Text-figures)

CONTENTS

I OUTLINED recently the origin of a number of tobacco, wheat, tomato, and other polyploid plants and described the behaviours of some practically constant, as well of some unconstant (segregating), allopolyploids

and autopolyploids, in a series of publications, and pointed out the causes for their behaviour (Kostoff, 1932–8*b*). There is no doubt that the allopolyploid forms *Nicotiana glauca* Grah.—*N. Langsdorffii* Weinm. and their progenies are the most interesting ones, from a cytogenetic and phylogenetic point of view, among the abundant material which I have accumulated during the last ten years upon polyploidy in connexion with interspecific hybridization. Describing their origin and behaviour I shall also discuss the evolutionary significance of such forms as might arise in nature.

Material and methods

Nicotiana glauca Grah. (syn. *N. arborea* Dietr.) is a perennial bush from Argentine. It has been transferred more recently to Australia, where at the present time it occupies large areas. In the Mediterranean zones of Europe it reaches a height of *ca.* 2–3 m., and its roots and the lower parts of the stems sometimes can over-winter when one covers them in autumn with soil. In our greenhouses it lives for many years and reaches a height of *ca.* 2–3 m. Stem—woody, branched; leaves with long petioles (Text-fig. 21); flowers 30 mm., yellow-greenish, formed at the top of the shoots (Text-fig. 23); pollen grains—white. The plant contains *ca.* 0·5–1·0 % alkaloid anabasine and *ca.* 3·5–4·00 % citric acid in form of various salts when grown in the Moscow region. The strains of *N. glauca* vary in respect to the size and shape of the flowers and leaves, anthocyanin content and the length of their vegetation periods. Comes (1899) included this species into the section *Rustica* of the genus *Nicotiana*.

N. Langsdorffii Weinm. is a herbaceous species from East Brazil. Some plants can over-winter in the greenhouse when good care is taken. In the field it may reach a height of *ca.* 80–90 cm. Stem—branched; leaves—sessile (Text-fig. 23); flowers—*ca.* 25 mm., slightly zygomorphic, yellow-greenish; pollen grains—violet-bluish. (This is the only *Nicotiana* species that has violet-bluish pollen like *Petunia violacea*.) Comes (1899) included this species into *Rustica* section. The flower colour is the only striking character that this plant has in common with the *Rustica* section. Habit of growth, leaf shape, flower shape, etc., resemble those of *Nicotiana alata* and *N. Sanderae*, the latter two species being typical representatives of *Petunioides* section. It crosses easily with these two species and the hybrids obtained are fully fertile. Lock (1909) and East (1928) were inclined to refer it to the *Petunioides* section. The latter author treated it rather as a "connecting link between *Rustica* and *Petunioides* sections" (p. 246). It should be mentioned here that the flower colour is not an

essential character. The studies by Anderson & de Winton (1931) and by East (1932) suggest a monofactorial difference, though more than one factor has also been suggested (Brieger, 1929). *Nicotiana Langsdorffii* has $n=9$ chromosomes like *N. Sanderae* and *N. alata*. Its chromosomes are homologous with those of these two species. Consequently, *N. Langsdorffii* should be included into *Petunioides* section.

Parallel with *Nicotiana glauca* and *N. Langsdorffii* species, I shall consider in this paper *N. Sanderae* (a horticultural plant) and *N. alata* (Uruguay) species, that were used for back-crosses of F_1 *N. glauca* × *N. Langsdorffii* hybrids. *N. alata* has probably participated in the origin of *N. Sanderae*. The strains of the latter are usually self-sterile and cross-fertile (i.e. highly heterozygous), quite often segregating *N. alata*-like types.

Cytological studies were carried out on paraffin preparations (permanent), aceto-carmine smear preparations and smear permanent preparations. Fixations used were: Bouin as modified by Allen and new modifications, strong Lewitzky's chrom-formol fixations, S. Navashin's chrom-formol acetic acid fixations, La Cour 2.BE, and Lewitzky's platinum chloride formalin fixation. Permanent preparations were stained by iron alum Heidenhain's haematoxylin, and by gentian violet iodine stains. Drawings were chiefly made by Abbé camera lucida, microscope Zeiss, oc. 20, obj. 90 (oil immersion), or Reichert, 12 comp. ocular × 12 oil immersion at the table level.

Determinations of the alkaloid and citric acid contents were carried out in Dr Shmuck's biochemical laboratory.

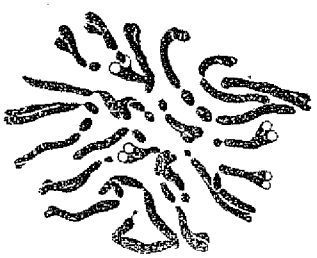
Cytology of the parents N. Langsdorffii *Weinm. and* N. glauca *Grah.*

Mitosis and meiosis of the parental forms were studied several times in various conditions. Some of the earliest studies were carried out about ten years ago when the author was working at Harvard University. Repeated investigations were also carried out in Sofia, Leningrad and Moscow. Extreme environmental conditions more easily disturbed meiosis but mitosis was also affected.

A. *Mitosis*. The procedure of mitosis was studied in both *N. glauca* and *N. Langsdorffii* plants.

N. glauca ($2n=24$, $n=12$). Somatic chromosome number in *N. glauca* was first determined by Goodspeed (1923, 1924). The same number was found later in this species by R. Clausen (1928), Christoff (1928), Kostoff (1930, 1934, 1935), Kostoff & Pavloff (1931), Sarana (1934), etc. *N. glauca*

is a tobacco species which has seven very long chromosome pairs out of twelve. In studying cytologically about forty tobacco species I have the impression that the longest chromosomes of the genus *Nicotiana* are present in *N. glauca*, though this is true for the longest only. In analysing the karyotype of *N. glauca* ten pairs were found with subterminal centromeres and two with submedial ones. One of the latter is satellite (Text-figs. 1, 2). *N. glauca* chromosomes stain much better in all kinds of preparations than those of the other *Nicotiana* species. It seems that *N. glauca* chromosomes absorb much more dyes than those of the other species. Chromosome alterations and doubling in *N. glauca* were induced



Text-fig. 1. Somatic plate of *N. glauca* ($2n=24$).

Text-fig. 2. Somatic chromosomes of *N. glauca*.

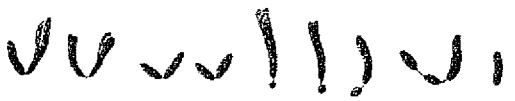
by acenaphthene treatments (Kostoff, unpublished) and by wounding (Pratassenya, 1935).

N. Langsdorffii ($2n=18$, $n=9$). The chromosome number of this species was studied by Goodspeed (1923, 1924, 1933), Vilmorin & Simonet (1928), Clausen (1928), Christoff (1928), Kostoff (1929, 1930*a*, 1934*a*, 1935*d*), etc. Goodspeed's first data were not quite decisive. His later studies and those by the other authors showed that *N. Langsdorffii* has $n=9$, $2n=18$. Christoff's statement that *N. Langsdorffii* as well as the other closely related species of the genus *Nicotiana* (*alata*, *Sanderae*) have $n=8$ and $2n=16$ is not correct. Polyploidy in *Nicotiana* is a frequent phenomenon, but $n=8$ has not yet been found. Gametic chromosome numbers found in *Nicotiana* are: 9, 10, 12, 16, 18, 20, 22, 24 and 32. In addition to these new allopolyploid and autopolyploid forms others have been produced with the following gametic chromosome numbers: 18, 20, 21, 24, 25, 32, 36, 40 and 48, the number 40 being derived from $16+24$ instead from 20.

These numbers show that *N. Langsdorffii* is one of *Nicotiana* species having the smallest chromosome number, like *N. alata*, *N. Sanderae*, and *N. bonariensis*.

In studying the karyotype of *N. Langsdorffii* the following chromosome types can be differentiated (Text-figs. 3, 4): (1) one long pair with submedian constriction, (2) one long pair with median constriction, (3) two small pairs with almost median constrictions, (4) three pairs with subterminal constrictions, one long and two medium, (5) one long pair with a secondary constriction (clearly visible only in some preparations), and (6) one very short pair having, so far as our preparations showed, most probably, terminal or almost terminal spindle fibre attachment (cf. Text-fig. 4 from left to the right).

Text-fig. 3. Somatic plate of *N. Langsdorffii* ($2n=18$).



Text-fig. 4. Somatic chromosomes of *N. Langsdorffii*.

Disturbances in the somatic chromosome number in *N. Langsdorffii* were induced by centrifuging (Kostoff, 1935*d*).

B. *Meiosis*. There is a large number of species in nature that have abnormal meiosis, but *N. Langsdorffii* and *N. glauca*, when developed and flowered in so-called "normal" conditions, had normal meiosis.

N. glauca. During diakinesis the smallest chromosomes had one or two chiasmata while the longer ones had 2, 3 and rarely 4 chiasmata. In one pollen mother cell (P.M.C.) during the diakinesis 27 chiasmata were counted, and in another one 23 chiasmata were observed, i.e. 2·08 per bivalent or nearly 2 chiasmata per bivalent. During the first meiotic metaphase I counted in one P.M.C. 18 chiasmata and in another one 21 (± 1), which gives at the average 1·63 chiasmata per bivalent. These numbers suggest that terminalization proceeds gradually from the one to the other phase.

Shoots with floral buds of *N. glauca* when covered with test-tubes which have acenaphthene crystals on the inside of the tube walls, the sublimating particles from acenaphthene induce abnormal meiosis. During the first metaphase bivalent chromosomes are not arranged on a regular equatorial plate, but occupy the place they have occupied during the diakinesis, though somewhat closer together. Then they divide without a complete terminalization and get spread abnormally into the cytoplasm in small groups. Each group, sometimes even single chromosomes, form a microspore, so that a large number (sometimes over twelve) of microspores are formed in each pollen mother cell during the tetrad stage. Such a cell reaction leads to formation of large percentage of abnormal pollen (30–100 %, depending on the quantity of the inductor) and large pollen with abnormal chromosome numbers.

N. Langsdorffii. This species has also regular meiosis under "normal" conditions. During diakinesis the longer chromosomes had somewhat more chiasmata than the shorter ones, as in *N. glauca.* In counting the chiasmata in four P.M.C., the following numbers were respectively obtained: 16, 18, 18, 20, which gives 2 chiasmata per bivalent. In counting the chiasmata in three P.M.C. during the first metaphase I found the following numbers: 16, 15, 12, i.e. 1·59 chiasmata per bivalent. These data indicate that terminalization in *N. Langsdorffii* proceeds as in *N. glauca.*

N. Langsdorffii floral buds treated with acenaphthene by the method with which *N. glauca* was treated reacted in the same way as in the latter species.

Abnormal meiosis in *N. Langsdorffii* was observed by the author in an intergeneric graft combination when *N. Langsdorffii* was used as a scion (Kostoff, 1930*d*). Meiotic irregularities in this case lead to production of chromosomal aberrants.

F_1 HYBRIDS *N. GLAUCA* × *N. LANGSDORFFII*

Hybrids of *N. glauca* × *N. Langsdorffii* were first recorded by Gärtner (1849), who stated that they were difficult to obtain. His hybrids were completely sterile; the flowers fell off several days after opening.

During the last twelve years I have several times carried out crosses between these two *Nicotiana* species, and have every year grown hybrids from this cross for various kinds of studies.

(a) *Crossability*

The crossability of *N. glauca* with *N. Langsdorffii* is very variable. It depends greatly on the individuality of the plants and on the environment in which the crosses are carried out. Some *N. glauca* plants cross more easily than others. It should be mentioned here that the *N. glauca* plants with which I worked were somewhat heterozygous in respect to some minute morphological characters (anthocyan, leaf index). It is possible that they have also differed in respect to certain biochemical characters. It is interesting to note that crosses made in spring and autumn were more successful than those made in summer (Table I).

N. glauca forms on the average about 800–850 ovules per capsule, while the largest number of hybrids obtained per capsule was 93. On the average, 28 hybrids per capsule were obtained from the cross *N. glauca* × *N. Langsdorffii*. The reciprocal cross usually fails, though I have been able to obtain it several times. In Table I are given some data from which one can judge of the crossability of this combination. The occasional failure of *N. Langsdorffii* × *N. glauca* cross is chiefly due to the slow growth of *N. glauca* pollen tubes in *N. Langsdorffii* styles. In the crosses *N. glauca* × *N. Langsdorffii*, the pollen tubes of *N. Langsdorffii* usually reach the ovary and fertilization generally takes place. Non-germination of the seeds from these crosses is chiefly due to slow growth of the hybrid embryos. Some of the young hybrids occasionally die at various stages of development.

(b) *Morphological appearance of F_1 hybrids*

The hybrids *N. glauca* × *N. Langsdorffii* appeared to be morphologically identical with those of the reciprocal cross *N. Langsdorffii* × *N. glauca*. At least I could not notice any significant difference. The majority of the hybrids usually grow normally until the time of florescence. Single plants, however, developed very abnormally, forming tumours, fasciations, and witches' broom-like malformations. Some *N. glauca* plants when crossed with *N. Langsdorffii* produced hybrids, the majority of which died at the seedling stage of development. Other plants produced approximately one-quarter dwarf and about three-quarters well-developed hybrids. Dwarf hybrids grew in the greenhouse *ca.* 30–35 cm., while the normal ones were *ca.* 90–120 cm. In the field, the dwarf hybrids grew about 30–40 cm. high, while the non-dwarf ones were *ca.* 140–160 cm. (150 cm. on the average). In 1935 I grew in Leningrad sixty-three F_1 hybrids *N. glauca* × *N. Langsdorffii* from a cross, fifteen of them were dwarfs and forty-eight developed normally. In

TABLE I

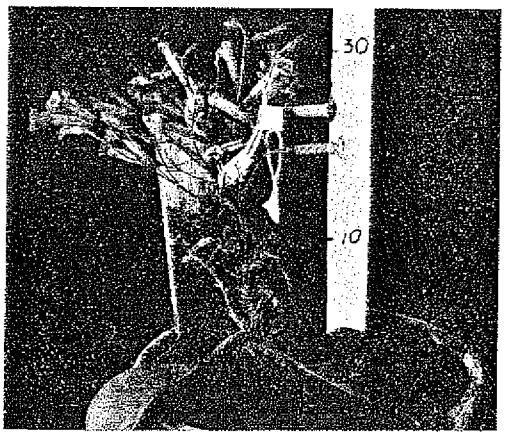
Crossability of N. glauca *with* N. Langsdorffii

Maternal plant	Paternal plant	Experiment carried out in	Date	Pollinated flowers	Capsules obtained	Germination
N. glauca	*N. Langsdorffii*	Boston, U.S.A.	1927, March–April	24	18	Seeds germinated
N. glauca	*N. Langsdorffii*	Boston, U.S.A.	1927, July	27	15	Seeds germinated only from three capsules
N. glauca, plant no. 5	*N. Langsdorffii*	Boston, U.S.A.	1928, April–May	15	15	Seeds germinated of each capsule
N. glauca, plant no. 7	*N. Langsdorffii*	Boston, U.S.A.	1928, June	15	8	Seeds germinated from five capsules
N. glauca, plant no. 5	*N. Langsdorffii*	Boston, U.S.A.	1928, June	15	3	Seeds did not germinate
N. glauca, plant no. 035–1	*N. Langsdorffii*	Sofia, Bulgaria	1931, May	10	9	Seeds of each capsule germinated
N. glauca, plant no. 035–2	*N. Langsdorffii*	Sofia, Bulgaria	1931, July	25	20	Seeds germinated only from seven capsules
N. glauca, plant no. 035–2	*N. Langsdorffii*	Sofia, Bulgaria	1931, October	25	24	Seeds germinated from each capsule
N. glauca	*N. Langsdorffii*	Leningrad, U.S.S.R.	1933, May	12	12	Seed germinated from each capsule
N. glauca (the same plant)	*N. Langsdorffii*	Leningrad, U.S.S.R.	1933, August	24	8	Seed germinated badly from each capsule
N. Langsdorffii	*N. glauca*	Sofia, Bulgaria	1931, June	50	1	Seed germinated
N. Langsdorffii	*N. glauca*	Sofia, Bulgaria	1931, September	38	9	Seed germinated only from two capsules

Remark. I am giving here only those of the crosses for which I have now exact data. This cross-combination, however, was carried out many more times without noting exactly the crossability.

1937 I grew fifty F_1 plants of the same combination in Moscow; fourteen of them were dwarfs. One dwarf plant is given in Text-fig. 5.

N. glauca usually has hairless stem and leaves (except on the main nerves, that appear in some plants), while the stem and the leaves of *N. Langsdorffii* are covered with small trichomes. F_1 hybrids were covered with small trichomes as in *N. Langsdorffii*. The latter parent has sessile leaves and blue (slightly violet) pollen, while *N. glauca* has petiolate leaves and white pollen. The shape of the leaves in F_1 hybrids is approximately intermediate in respect to those of the parental forms (Text-fig. 21). The hybrids have also blue (slightly violet) pollen, but the colour of the pollen in F_1 hybrids is somewhat diluted.



Text-fig. 5. F_1 dwarf hybrid *N. glauca* × *N. Langsdorffii*.

F_1 hybrids form, as a rule, hereditary non-parasitic tumours on the stems, roots, shoots and occasionally on the leaves, usually when the plants became old, i.e. after the florescence period (of the main stem). When the seedlings are raised in April, the hybrids usually begin to flower in July, while tumours begin to appear in August, September and October. All F_1 hybrid plants formed tumours, whenever and wherever they were raised. I grew some F_1 hybrids in Boston (Bussey Institution, Harvard University, 1927–9), in Sofia (Bulgaria, 1930–2), in Leningrad (Botanical Garden, 1932–5), and in Moscow (1935–8). They all formed tumours at all these localities. I grafted shoots from F_1 hybrids on the parental forms and vice versa. The hybrid tissues (shoots) formed tumours, no matter whether grown as scion or as stock, while the parental parts did not show the symptoms. All attempts to isolate parasites (bacteria or

fungi) or to inoculate other tobacco species were unsuccessful. Consequently the conclusion was drawn that these tumours are heritable non-parasitic. Allopolyploids produced by chromosome doubling in F_1 hybrids also form the same kind of tumours and this character is transmitted to the subsequent generations.

It should be mentioned that certain F_1 plants occasionally form tumours and fasciations even before the beginning of the florescence period. When hybrid seeds are sown in September in the greenhouse, the hybrid usually begins to flower next year in April or May. Such hybrids usually form tumours before flowering, being so to say "old" (Text-fig. 6).

Tumours are formed on the stems: (1) where, accidentally, the cortex is wounded, (2) at the place of leaf abscission, (3) where new branches start to develop (tumours are formed instead of differentiated branches), (4) at any place on the stem and side branches (cambium begins to divide continuously uncontrolled at various places and produce undifferentiated cells, thus forming tumourous malformations). Tumours formed on the stems sometimes reach the size of a walnut.

Leaves formed tumours most frequently at the place of small or large injuries, especially when the latter have involved places with active cambial tissue (large veins). Tumours formed on the leaves reach sometimes a size of a pea seed.

The largest tumours are formed on the stem-root regions, just at the place that touches the surface of the soil. This is the place where most abrupt changes take place (moisture or drought). The tumours formed in this region occasionally reach the size of a small chicken egg.

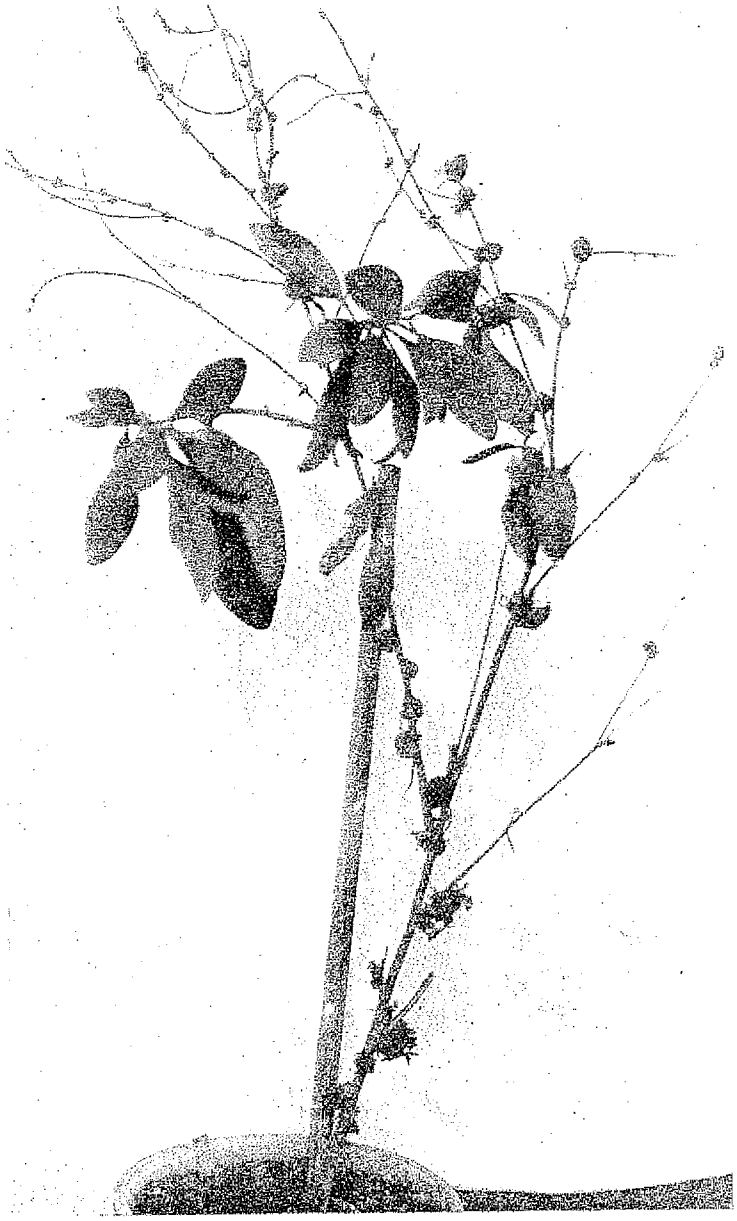
Tumours were formed by *N. glauca* × *N. Langsdorffii* as well as by the reciprocal hybrid. There are no noticeable morphological differences between those formed by the former and those formed by the latter.

(c) *Cytology of F_1 hybrids* N. glauca × N. Langsdorffii

(1) *Mitosis.*

Somatic chromosomes of F_1 hybrids were studied in the root tips. Some chromosome counts were also made in the somatic tissues of the floral buds and in the tumours.

In the root tips, as a rule, 21 chromosomes were found, representing the sum of the haploid sets of the parental species. Most of the parental chromosomes were easily identified in F_1 hybrids (Text-figs. 7–9). The *N. Langsdorffii* chromosome with the secondary constriction was rarely identified. Its secondary constriction was revealed with difficulties even



Text-fig. 6. F_1 *N. glauca* × *N. Langsdorffii* forming numerous large tumours after the flowering period.

in the parental species. The satellite chromosome of *N. glauca* was easily identified (Text-fig. 10).

For studying mitosis in F_1 hybrids as compared with the parental forms, longitudinal sections were made through the root tips. In 216 anaphases from an F_1 hybrid, four abnormal mitotic figures were found. Three anaphases had laggards on the spindles and one had a chromatin bridge. In 195 anaphases in *N. glauca* root tips and 204 anaphases in *N. Langsdorffii* no abnormalities were noticed. These data indicate that

Text-fig. 7. Text-fig. 8. Text-fig. 9.

Text-figs. 7, 8, 9. Somatic plates of F_1 *N. glauca* × *N. Langsdorffii* hybrids.

Text-fig. 10. Satellite chromosome from the root tips (left), from the somatic tissues of the floral buds (in the middle) and from the tumours (right) of F_1 hybrids. Note the contraction.

the frequency of abnormal mitosis in the hybrid was higher than in the parental species. They also show that about one cell out of fifty may have abnormal karyotypes. The fact that somatic plates with 21 chromosomes were usually found shows that the cells with the abnormal karyotypes cannot as a rule compete with the normal cells, having a lower frequency of cell division than the cells with normal karyotypes.

Chromosome number in the root tips was studied in transverse sections. I found usually 21 somatic chromosomes, but sometimes other chromosome numbers were counted (22, 23, 28, 42, etc.), though very rarely. Such deviations were not found in the parental species.

Abnormal mitosis was also found in the somatic tissues of the floral buds, but no exact counts were carried out. The chromosomes in the soma of the floral buds appeared somewhat shorter than those in the root tips (Text-fig. 10).

The chromosome number in the tumours is usually the same as that in the other somatic cells, namely, 21. Tetraploid cells and areas with 42 chromosomes were relatively rarely found. I have found them, most frequently, near the necrotic regions. These observations indicate that the polyploidy in tumours is a secondary phenomenon, and cannot be included in the chain of reactions that condition tumour formations (cf. Kostoff, 1930*b*, 1931*b*, 1933*a*, *b*, 1935*c*; Kostoff & Kendall, 1930, 1933).

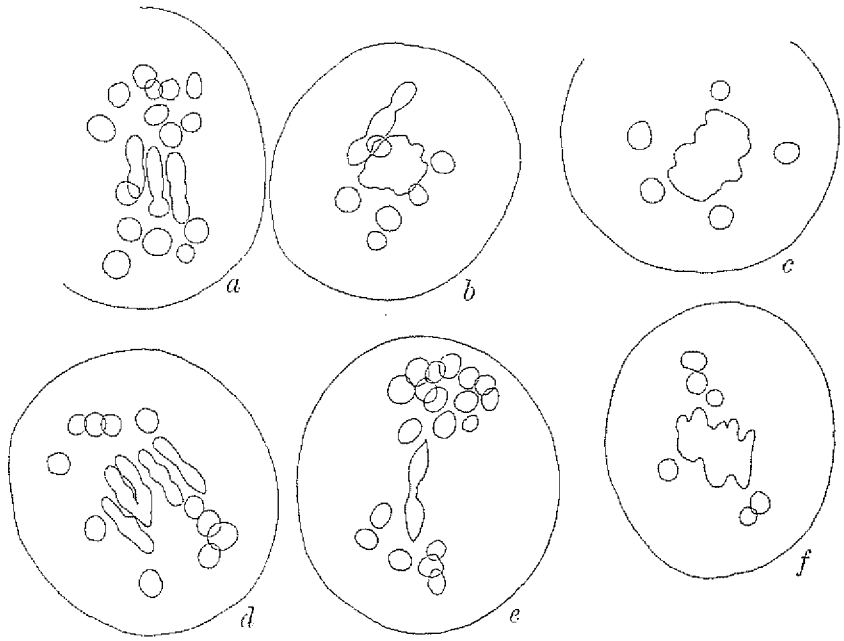
I cannot maintain now with certainty that there is a definite causal connexion between the polyploidy and necrosis, since the occurrence of the polyploid cells and tissues, near the necrotic regions, is rather a tendency than a rule. It is also difficult to decide whether the necrosis is more likely to occur in the polyploid tissues, or whether the "necro-hormones" (*sensu* Haberlandt) and other products of the death cells are the responsible agents for the induction of polyploidy.

In summer time when the plants assimilate intensively, the tumours contain a large amount of starch, while in winter at certain periods one cannot find starch in the tumour cells.

Tumour cells represent rapidly dividing non-organized meristematic cells which expand, sometimes enormously without being differentiated. They are usually rich in cytoplasm and have small but numerous vacuoles, thus much resembling the cells in plant galls situated in the proximity of the parasites (Kostoff & Kendall, 1929, 1930; Kendall, 1930). In the tumour cells one can find occasionally crystals of calcium oxalate. Tumour cells often have larger nuclei and a larger number of nucleoli. This is especially true for tissues near the necrotic regions, where one often finds cells with abnormally deformed nuclei. Cytolysis in the necrotic regions is sometimes accompanied by disappearance of the nuclear membranes (nucleolysis). One finds that the prochromosomes stain better in the nuclei in the resting cells of tumours, especially in the cells situated near the necrotic regions, than in the other cells of F_1 hybrids. The prochromosomes seem to correspond to the heterochromatic regions of the chromosomes.

Aneuploid cells were very rarely found in the tumour tissues. I was able to count their chromosomes only in a few instances. They were 23, 27, 30, etc., instead of 21 or 42. They obviously result from abnormal

mitosis. Abnormal mitoses were also found in the tumours. They occurred here somewhat more frequently than in the root tips. But since tumour cells have as a rule 21 somatic chromosomes, i.e. the normal somatic chromosome number, we have no reason to assume that polyploidy or heteroploidy (involving whole chromosomes) are responsible for the tumour formations. On the contrary, it seems more probable that the mitotic abnormalities which lead to formation of polyploid and aneuploid



Text-fig. 11. Meiosis in F_1 hybrids *N. glauca* × *N. Langsdorffii* with one (*e*) and more than one bivalents; with one trivalent (*b*, *d*); with one heteromorphic (one larger and one smaller component chromosome) bivalent and two almost homomorphic (*a*); and with various number of univalents.

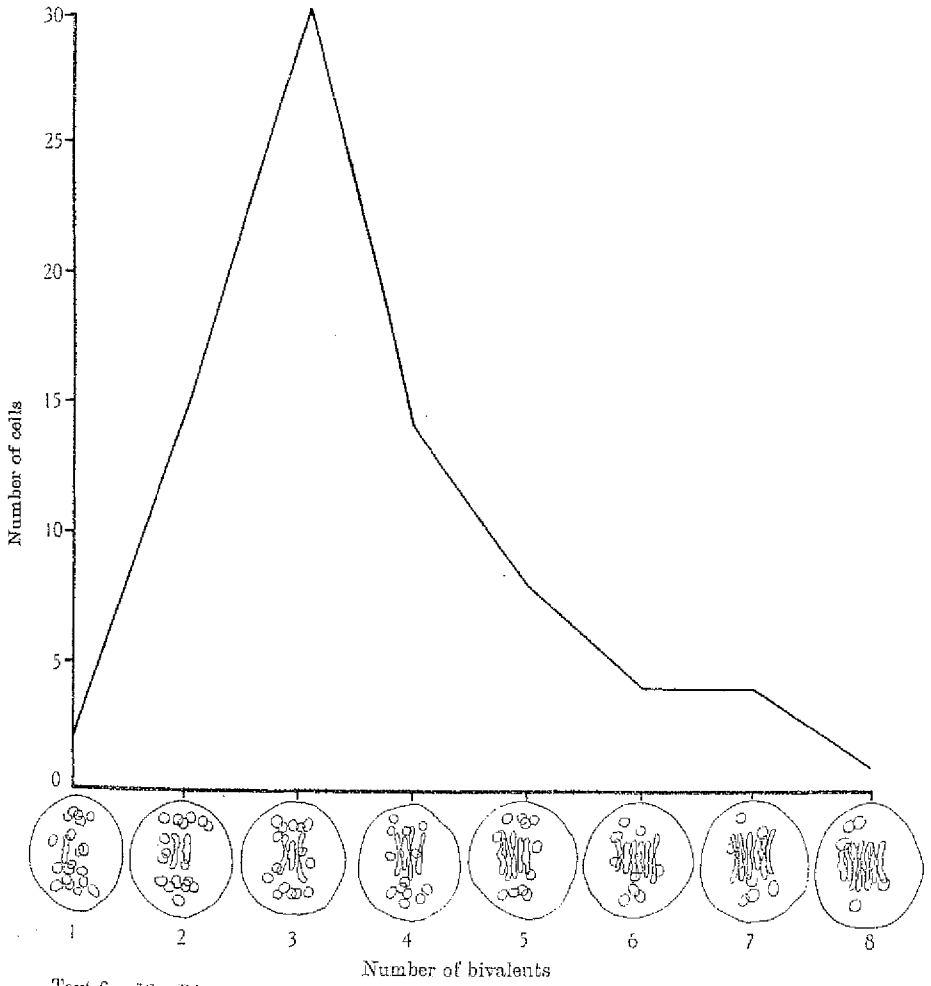
cells are rather the consequences of the cause or causes conditioning tumour formation.

(2) *Meiosis.*

Meiosis was studied in the pollen mother cells (P.M.C.). The first metaphase and the later stages were more thoroughly investigated. During diakinesis various numbers of univalents and bivalents were found. Bivalents had most frequently terminal chiasmata, but subterminal were also observed.

During metaphase very variable numbers of bivalents were found. They varied in different plants, in different floral buds and in different

cells. F_1 hybrids studied in America and Bulgaria had on the average many more bivalents than those grown in Leningrad and Moscow. The number of bivalents seems to depend on the genotype and on the en-



Text-fig. 12. Diagram showing the frequency of the number of bivalents in the pollen mother cells (P.M.C.) during the late first metaphase of the F_1 hybrid *N. glauca* × *N. Langsdorffii*. The cells on the abscissa were drawn first from the preparations and then semidiagrammatically redrawn.

vironmental conditions. Dwarf F_1 hybrids, *N. glauca* × *N. Langsdorffii*, had on the average fewer bivalents than the normally developing ones. Table II gives the number of cells having one, two, three, etc., bivalents (cf. Text-figs. 11, 12; Pl. IV, figs. 12–15).

The data are not very exact for the following reasons: (1) Cells with trivalents were excluded. They seem to appear somewhat more frequently in the dwarfs than in the normally developing F_1 hybrids. About fifteen P.M.C. from the dwarfs having about 5–9 bivalents were also discarded, since I was not able to determine exactly the number of the bivalents. About twenty such cells were also discarded from the normally developing hybrids. If one takes these cells into account, the curve (Text-fig. 12) would be more symmetrical. It is evident from the data (Table II) that dwarfs have most frequently 3 and 4 bivalents. Considering the discarded cells, it seems more probable that they have most frequently 4 bivalents. Sister plants developing normally have most frequently 6 bivalents, but a similar correction for discarded cells should also be introduced here, so that 6 and 7 bivalents are probably most frequent in these hybrids. I grew some F_1 hybrids in America which had most frequently 9 bivalents and 3 univalents (Kostoff, 1930*a*).

TABLE II

Bivalent frequency in F_1 N. glauca × N. Langsdorffii *hybrids*

	Bivalents										
Number of P.M.C.	1	2	3	4	5	6	7	8	9	*n*	*M*
Dwarf:											
F_1 hybrids	2	15	30	14	8	4	4	1	—	78	3·5
Normal:											
F_1 hybrids, sister plants of the dwarfs	—	2	8	15	17	19	13	12	14	100	6·0

During the first meiotic metaphase one trivalent chromosome group was sometimes observed. In side view they were V-like or I-like (Text-fig. 11). Another peculiarity, quite often observed, was the conjugation of morphologically different chromosomes (allosyndesis), i.e. a long chromosome forming chiasma with a short one (Text-fig. 11; Pl. IV, figs. 12–14). It is quite possible that a short chromosome from one species (probably *N. Langsdorffii*) synapsed during the early prophase with a long one from the other parental species (probably *N. glauca*) and, crossing-over, they formed an asymmetric bivalent with unequal component chromosomes. The course of meiosis in *N. Langsdorffii* haploids (Kostoff, 1929, 1938*i*) supports the idea that bivalents result from allosyndesis, since haploid *N. Langsdorffii* had usually asyndesis; one or two autosyndetic bivalents being rarely found.

The asymmetric chromosomes in F_1 *N. glauca* × *N. Langsdorffii* cannot be interpreted as an autosyndetic product of *N. glauca* chromosomes,

since in the F_1 hybrid *N. suaveolens* ($n=16$) × *N. glauca* ($n=12$) asyndesis was usually found and bivalents only occasionally, the latter being rather a product of allosyndesis.

Bivalents found in the F_1 hybrids *N. glauca* × *N. Langsdorffii* were usually held by single terminal or subterminal chiasmata during the first metaphase, thus the average numbers of chiasmata per cell in the F_1 hybrids, mentioned in Table II, would respectively have *ca.* 3–4 and 6–7 chiasmata per cell, which means that there is a very significant reduction in respect of those of the parental forms.

Considering these data and the great variability in the number of the bivalents (1–9) one can conclude that *N. Langsdorffii* chromosomes have segments homologous with some segments of *N. glauca* chromosomes. These segments obviously vary in size, but the occurrence of 1–9 bivalents indicates that the homologous parts are not large (in length).

The formation of chiasmata in the homologous segments conditions exchange of parts between *N. glauca* and *N. Langsdorffii* chromosomes, which leads further to recombination of characters.

The occasional occurrence of a trivalent group of chromosomes during the first meiosis in the F_1 hybrids suggests that one chromosome of the one parent has homologous segments to two different chromosomes of the other parent. It should also be mentioned that haploid *N. Langsdorffii* may form a trivalent group autosyndetically, but this trivalent group occurs very rarely; therefore it does not seem to be the only one observed in the F_1 hybrids. Trivalency increases the diversity of the new types of chromosome resulting from "cross-over" exchanges in the F_1 hybrids (Text-fig. 34). In addition to the exchanges that occur in the homologous segments between partially homologous chromosomes *N. glauca* and *N. Langsdorffii*, those should also be considered that might occur among the chromosomes of the one and of the other parent following autosyndesis.

The occasional occurrence of one or two bivalents in haploid *N. Langsdorffii* suggests this idea. Autosyndesis might result from conjugation between small homologous segments (duplications) or between the heterochromatic regions of the non-homologous chromosomes (cf. Kostoff, 1938*b*, *g*, *h*). Conjugations between heterochromatic regions of the non-homologous or partially homologous chromosomes in F_1 hybrids might also occur and lead to chiasma formation and exchange of parts. This also increases the number and the diversity of exchanges.

The formation of a variable number of bivalents and univalents, and occasionally trivalents, in F_1 hybrids during the first meiotic division

further conditions various kinds of meiotic abnormality characteristic for structural hybrids and for hybrids originating from parents with unequal chromosome numbers. I shall note here, however, two characteristic phenomena that are of importance for the progenies of F_1 hybrids *N. glauca* × *N. Langsdorffii*.

(1) The univalent chromosomes usually do not divide during the first meiosis but get spread all over the spindle and very often lead to formation of restitution nuclei (non-occurrence of the first meiosis). In the haploid *N. Langsdorffii* ($n=9$), I found that in 183 P.M.C. of one floral bud only 14 univalents had divided during the first meiosis, which means that about 7·5 % of the P.M.C. have one divided univalent chromosome; or one P.M.C. out of thirteen has one divided univalent chromosome which means that only 0·85 % of the univalents divide during the first meiosis.

TABLE III

Microspores	Monads	Dyads	Triads	Tetrads	With more than four microspores
Number of P.M.C.	3	48	8	63	15

In studying 110 P.M.C. of another floral bud I found that only one univalent chromosome was divided in a P.M.C., which means that 0·1 % of the univalent chromosomes have divided. In the F_1 hybrid *N. glauca* × *N. Langsdorffii* I studied a much smaller number of cells for division of univalents than in the haploid *N. Langsdorffii*, viz. fifty-one cells, and I found two cells in which the sum of the chromosomes during the second metaphase was 22 instead of 21, which means that in these two cells two univalents have divided during the first meiosis. The percentage for the hybrid is *ca.* 4, i.e. about one cell out of twenty-five has a univalent chromosome that has divided during the first meiosis. The additional chromosome has resulted from a divided univalent because: (*a*) fragments that might originate following crossing-over in inverted regions during the first meiosis in reality do not get formed (absence of bridges and fragments), and (*b*) dividing univalents during the late anaphase were occasionally found on the spindle.

(2) The formation of restitution nuclei during the first meiosis conditions the appearance of all chromosomes (somatic number) in one plate during the second division, which further leads to formation of dyads. When a second division of such nuclei fails, monads are formed. The latter occurred rarely. In one F_1 hybrid I counted the following kinds of microspores formed (Table III).

These data show that the hybrid forms about 2 % monads and about 35 % dyads. Dyad formation is greatly influenced by the environmental

conditions. In autumn, when at night the temperature falls down to 8–6° C., the percentage of the dyads increases. It also increases in hot summers, when the temperature in the greenhouse rises above 40° C. during the day and the plants are not sufficiently watered. The percentage of dyads also depends on the genotype. At the same time when I counted in one F_1 hybrid *ca.* 35 % dyads, in another hybrid of the same cross-combination (its mother plant, *N. glauca*, was not the same) I counted about 17 % of dyads.

The percentage of viable pollen in F_1 was studied in connexion with the percentage of dyads and monads. In studying the P.M.C. of a hybrid during the tetrad stage, the following data were obtained (Table IV).

TABLE IV

Microspores	1	2	3	4	5 and more
P.M.C. studied	1	24	10	83	23
Pollen calculated	1	48	30	332	*ca.* 125

The total number of the P.M.C. studied was 141. They should give about 536 pollen grains.

In studying the percentage of the pollen grains of the same plant that stained deeply red in aceto-carmine preparations (the viable pollen) I found in one flower 7·5 %, in another 8 %, and in a third one about 8·5 %. The average percentage of viable pollen for this plant was *ca.* 8 %. The calculated percentage of the pollen formed from monads and dyads is about 9, i.e. somewhat larger than the percentage of the viable pollen but very near to it.

These data strongly suggest that the viable pollen grains formed by the F_1 *N. glauca* × *N. Langsdorffii* hybrids are those originating from dyads, and probably those of monads. In other words, the viable pollen should have most frequently the somatic chromosome number, i.e. 21. The diameter of the viable pollen formed by the F_1 hybrids is equal to the diameter of the pollen formed by the amphidiploids *N. glauca—N. Langsdorffii*. This is another argument in favour of the above postulate (cf. Table XIV).

The small amount of very large pollen formed by the F_1 hybrids (shown in Table XIV) probably originated from monads. The amphidiploids did not form such large pollen. Meiosis in the embryo-sac mother cells proceeds in the way as in the P.M.C.

Gametes originating from dyads having 21 chromosomes should not be genetically equal since chromosome conjugations and chiasma formations (crossing-over) takes place during the meiosis. Direct evidence for this

was supplied by crossing F_1 hybrids back to *N. Langsdorffii* ($n=9$). The majority of the hybrids thus obtained were "triploids" having one genom *N. glauca* (12) and two genoms *N. Langsdorffii*, i.e. 30 somatic chromosomes. These "triploids", having 30 somatic chromosomes, were not morphologically equivalent. Their differences are due to the chromatid exchanges that occur during the meiosis in F_1 hybrids (cf. Kostoff, 1934*b*, 1935*b*).

F_1 hybrids *N. glauca* × *N. Langsdorffii* were self-sterile, even though raised under various environmental conditions and artificially selfed. The percentage of viable pollen is sufficient to produce a few seeds if the pollen tubes reach the ovary. But this alone cannot secure seed production. It is also very doubtful whether the pollen tubes of this hybrid can reach the ovary on selfing.

I self-pollinated six flowers from this hybrid and fixed the styles 3, 5 and 7 days after selfing. The last two styles were taken from the flowers when they dropped from the hybrid, which they usually do without withering. In studying the pollen-tube growth in these styles I found in the first two styles that the ends of the several pollen tubes penetrating the style had reached the first third of the style. In the styles fixed 5 days after the self-pollination the longest pollen tubes had reached to about the middle of the style, and in those fixed 7 days after selfing, they had reached about the second third of the style.

As compared with the parental species and the *N. glauca*—*N. Langsdorffii* amphidiploids the pollen tubes of the F_1 hybrid were thicker than those of the parental species and about as thick as those of the amphidiploids. Thick pollen tubes with somatic chromosome number grow slowly, and usually cannot reach the ovary of the diploid form (cf. Kostoff, 1934*c*, 1938*c*, *j*; Kostoff & Prokofieva, 1935).

Even if a few pollen tubes reached the ovary, there is a very small chance of one entering an ovule with a viable egg cell. Again, if a viable zygote were formed it is improbable that it alone would suffice to induce the necessary stimulation for prevention of the capsule abscission. From data derived from back-cross experiments the lowest number of seeds necessary for this is three viable ones, or two viable and several swollen but non-viable.

The above studies show the causes for the self-sterility of the F_1 hybrid.

BACK-CROSSES

If one crosses F_1 hybrids *N. glauca* × *N. Langsdorffii* back to the parental species or to a third species and the pollen tubes of these species reach the ovary of the F_1 hybrids, it is possible that almost each of the ovules which contains viable egg cell can be fertilized. This means, that in back-crosses almost all viable egg cells can be fertilized. If the zygotes, thus produced, were viable, and if the embryos, thus formed, can grow further, one can obtain germinating seeds and hybrids from the back- and triple crosses.

I crossed F_1 hybrids *N. glauca* × *N. Langsdorffii* to the parental species and to *N. Sanderae* and *N. alata* at various periods of the year.

Pollen tubes of *N. Langsdorffii*, *N. Sanderae* ($n=9$) and *N. alata* ($n=9$) easily reach the ovaries of the F_1 hybrids, while the pollen tubes of *N. glauca* rarely did so.

When the first flowers at the beginning of the florescence period were crossed, no seeds were obtained. A few seeds were obtained when the F_1 hybrids were crossed at the end of their florescence period in the autumn, and next year in early spring when they begin to flower for a second time. In crossing sixty-three flowers of F_1 hybrids to *N. glauca* no seeds were obtained, the flowers usually dropping. When F_1 hybrids were pollinated with pollen from *N. Langsdorffii* seeds were obtained several times. In crossing about 90–100 flowers of F_1 hybrids at the beginning of the florescence period (end of June and July) to *N. Langsdorffii* no seeds were obtained. In crossing fifty-four flowers at Bussey Institution at the end of the flowering period eighteen capsules were obtained. Each capsule had at least two or several (up to seven) germinating seeds, and several large but shrunken non-germinating ones. Pollinating about thirty flowers in autumn at Sofia University in 1931 I obtained about ten capsules from which plants were raised. Similar data were also obtained from the crosses carried out in the winter of 1931–2.

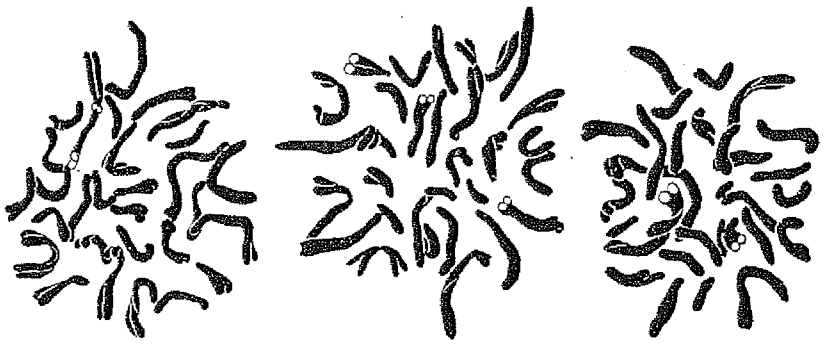
From the first series only seven plants were studied cytologically. Three of them had 30 somatic chromosomes (12+9+9), one had 29, one 32, one 21 and one had 20 chromosomes. The first three plants originated from an unreduced egg cell having the whole somatic chromosome set of *N. glauca* and two sets of *N. Langsdorffii* (cf. Kostoff, 1930*a*).

From the second and third series of crosses, plants were grown and studied cytologically. Eighteen plants from the second series all had 30 somatic chromosomes. From the third series 35 plants were grown. One of them was an amphidiploid, two were aberrants having 31 chromo-

somes, and all the other 32 plants from the back-cross had 30 somatic chromosomes, i.e. they originated from fusions of unreduced egg cells having the somatic chromosome number (21) and normal sperms of *N. Langsdorffii* (Text-figs. 13–15).

The plant with 20 chromosomes, "triploid" plants ($2n=30$), i.e. mono-*glauca*—di-*Langsdorffii* hybrids and the amphidiploid one were the most interesting forms, therefore I shall consider here some of their important characteristics and behaviour.

The plant with 20 chromosomes (No. 81) originated from an egg cell of the F_1 hybrid having 11 chromosomes and a normal sperm of *N. Langsdorffii*. Morphologically it was deformed, forming small asymmetric leaves with very uneven surface. The flowers were also small, often



Text-fig. 13. Text-fig. 14. Text-fig. 15.

Text-figs. 13, 14. Somatic plates of two different trigenomal hybrids mono-*glauca*—di-*Langsdorffii* ($2n=30$).

Text-fig. 15. Somatic plate from a hybrid (*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii* with 31 chromosomes (plant no. 346/34).

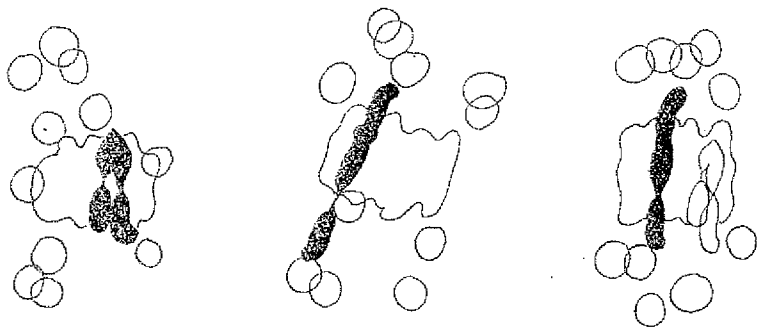
asymmetric, usually with four instead of five petals and an uneven surface as in the leaves. At the top of some branches this plant formed quite normal somewhat larger flowers, resembling those in F_1 hybrids about one month after the formation of the first abnormal flowers. The anthers of the deformed flowers, formed at the beginning of the florescence period, did not open. When the anthers with "matured" pollen grains after the opening of the flowers were opened by a needle and studied in aceto-carmine preparations all the grains were found to be abortive. The well-developed flowers, on the contrary, formed a large amount of pollen, of which only about 15–20 % was viable. Small and deformed flowers were sterile both after selfing and after crossing with *N. Langsdorffii*, while the larger ones (normally developed) set a small amount of germinating seeds after self-pollination. The morphology of the leaves

and the flowers with uneven surfaces, as well as the behaviour of the small and large flowers suggested abnormal mitosis for the hybrid no. 81 with 20 somatic chromosomes. In studying thirty root tips, twenty-nine had consistently 20 chromosomes and one was a chromosomal chimera having 20 and 40 chromosomes. In studying floral buds of the branches with small and large flowers, I found that the sum of the chromosomes in those forming small deformed flowers during the second division was 20, while the sum in the larger ones, that formed some viable pollen, was 26. One flower had altogether 40 chromosomes during the second division. The chromosome number counted in the pollen mother cells during the second metaphase, corresponds to that in somatic tissues of the floral buds. Abnormal mitosis with laggards was observed in the floral buds taken from branches that formed abnormal flowers. These observations showed that hybrid no. 81, obtained from the back-cross (*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii*, was a composite chromosomal chimera having parts with 20, 26 and 40 chromosomes. I found in this plant tissues with such chromosome numbers, but it was not excluded that other tissues with other chromosome numbers were also formed. The uneven surface of the leaves suggests very strongly that some groups of the cells divide more frequently than the others. It is possible that various groups of cells had unequal chromosome numbers. These observations show that hybrids may "mutate" more frequently in respect of the number of the chromosomes than the pure species (cf. Kostoff, 1930*a*, 1935*b*, 1938*c*). The behaviour of this plant and the observations made on other *Nicotiana* hybrids suggested that somatic mutations in hybrids, chromosomal and genic, being more frequent than in parental species, might be responsible in certain cases for the dying off of the hybrid embryos at various stages of development, as well as for survival of single embryos out of many thousands. Such cases were often observed in *Nicotiana* hybridization (Kostoff, 1935*h*, 1938*c*).

Mono-*glauca*—di-*Langsdorffii* forms, originating from unreduced egg cells of the F_1 hybrids and normal sperms of *N. Langsdorffii*, though having all exactly 30 somatic chromosomes, were not morphologically uniform. They differed in respect of size, time of flowering, leaf shape and size, flower size and shape, position of the stigma in respect to the anthers, etc. They also differed in respect of their meiosis and fertility.

Meiosis was studied in five plants. One of them formed one or two trivalents in almost all P.M.C. The number of the univalents varied from 9 to 12. The other four plants also formed one trivalent occasionally, rarely two (Text-fig. 16). They formed 10–14 univalents. The first plant

formed a large number of restitution nuclei and dyads (18–39 %). It also formed occasionally monads. Another triploid hybrid studied in 1929 formed a very large percentage of dyads. A photomicrograph of them was given in an earlier publication (Kostoff, 1930*a*, p. 133). "Triploids" formed a very variable percentage (8–50) of viable pollen. The latter were unequal in size. The largest pollen often germinated abnormally with branched pollen tubes, with two, or even with three pollen tubes. Most of the "triploids" were usually self-sterile, though some of them set a few capsules at the end of their flowering period, i.e. end of August and September. The largest numbers of seeds found in the capsules was thirty-six. Some did not set any seeds. Since the "triploids" obtained from the back-crosses (*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii* often formed restitution nuclei and dyads, it was supposed that if they could



Text-fig. 16. First meiosis in the P.M.C. of a trigenomal back-cross hybrid (*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii* ($2n = 30$). Note trivalents and univalents.

be crossed with *N. glauca*, amphidiploids *N. glauca—N. Langsdorffii* would be produced. An egg cell of the triploid back-crosses which has originated from a restitution nuclei should have two whole genoms of *N. Langsdorffii* (9/9) and one whole genom of *N. glauca* (12). If we could add one more *N. glauca* genom by crossing the "triploid" to *N. glauca*, an amphidiploid should arise with 9/9 *Langsdorffii* + 12/12 *glauca* chromosomes. In this case the dyad egg cells should not be alike since crossing over takes place in the trivalent groups as well as between some *N. Langsdorffii* reorganized chromosomes by chiasma formation during the meiosis in F_1 hybrids.

The latter reorganization accounts for the morphological differences and the differences in behaviour between the "triploids" having all exactly 30 chromosomes. I pointed out before (1934) that in a series of F_1 *Nicotiana* hybrids crossing-over takes place between allosyndetic

bivalent chromosomes; therefore the dyads, formed by these hybrids, give rise to morphologically different plants when crossed back to homozygous parent plants or to a homozygous third species.

"Triploids" (*N. glauca* × *N. Langsdorffii*) × *Langsdorffii* (mono-*glauca* —di-*Langsdorffii*) were crossed back to *N. glauca* during the whole florescence period. In crossing 324 flowers only six capsules were obtained. It was found that *N. glauca* pollen tubes do not usually reach the ovary of the triploids. Out of these six capsules only twenty-four visibly normal seeds were obtained from which fifteen plants were grown. Three of them died before the beginning of flower formation. One of them had about 72 somatic chromosomes, two had 42 chromosomes, i.e. they were amphidiploids, three had 42 ± 1 (they probably were amphidiploids too), and the others were chromosomal aberrants having less than 40 chromosomes. The plant that had about 72 somatic chromosomes probably originated from fusion of a monad egg cell in which both meiotic divisions have failed (60 chromosomes with a normal sperm of *N. glauca* (12)). It was partially fertile, thus having three *N. glauca* genoms and four *N. Langsdorffii* (tri-*glauca*—tetra-*Langsdorffii*).

The amphidiploid which was produced by crossing F_1 hybrid *N. glauca* × *N. Langsdorffii* to *N. Langsdorffii*, probably originated parthenogenetically from an egg cell in which both meiotic divisions failed to occur. It is probable that its parthenogenetic development was stimulated by the *N. Langsdorffii* pollen tube. Morphologically it was much more like the F_1 hybrids except that it was more robust, having broader and coarser leaves and larger flowers than those obtained in crossing the "triploids" to *N. glauca*. The latter were not quite alike as one might expect, since the egg cells from which they originated could not have been alike, the differences being conditioned by crossing-over between *N. glauca* and *N. Langsdorffii* chromosome segments during the meiosis in F_1 hybrids and in the back-crosses.

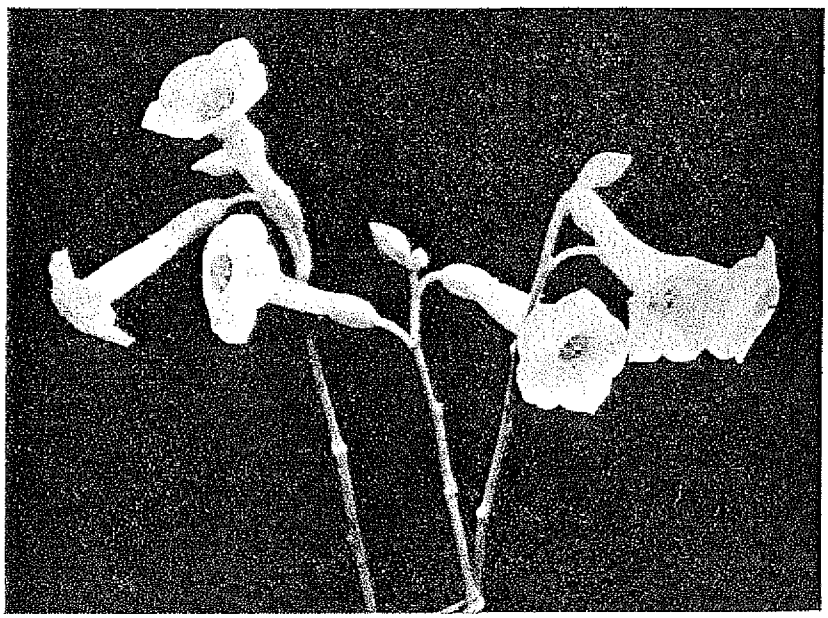
It should be finally noted that almost all plants obtained from the primary back-crosses (*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii* and from the secondary back-crosses (*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii* [× *N. glauca*] formed tumours, some earlier, others later. I have found only two aberrant plants from the primary back-cross that did not form tumours during the autumn in 1933 and in spring 1934. At least, I have not noticed on these two plants tumour-like malformations or fasciations.

Many attempts were made to cross *N. glauca* and *N. Langsdorffii* with pollen of F_1 hybrids, but no seeds have yet been produced from such crosses.

Parallel with the crosses (*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii* with *N. glauca* back-crosses were also made, using *N. glauca* as maternal plant. In crossing about fifty flowers only one capsule was set from the seed of which seven plants were raised. Three were studied cytologically. Two plants were chromosomal aberrants, while one of them had 42 chromosomes, i.e. it was an amphidiploid. The latter plant was broken at an early stage of development and it died before reaching maturity.

Triple crosses

The hybrids *N. glauca* × *N. Langsdorffii* were crossed to several other species in order to obtain triple hybrids for studying in them the chromosome behaviour and its further sequences (Text-figs. 17–19). Most interest-

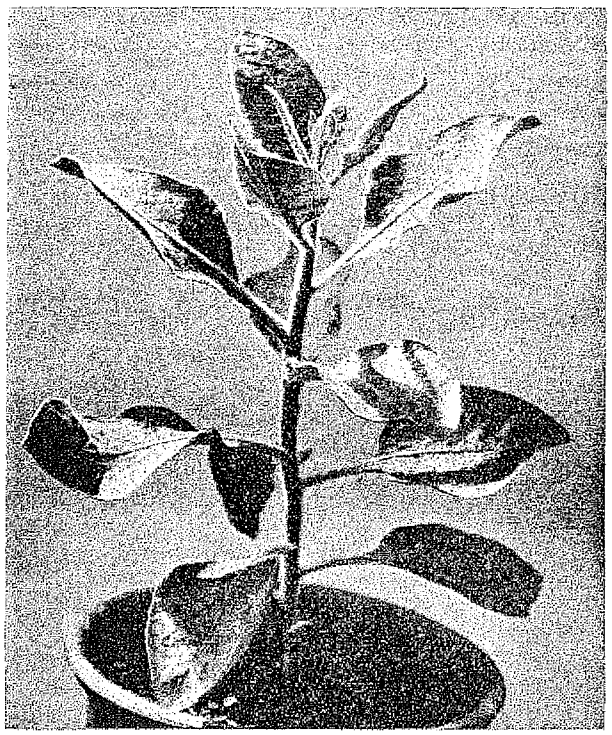


Text-fig. 17. Trigenomal triple hybrid (*N. glauca* × *N. Langsdorffii*) × *N. alata* ($2n=30$).

ing triple hybrids, obtained were (*N. glauca* × *N. Langsdorffii*) × *N. Sanderae*. I shall consider here only this triple hybrid. In producing triple hybrids from this cross-combinations, F_1 hybrids were crossed without castration. On the contrary, they were first purposely self-pollinated and then were pollinated with pollen from *N. Sanderae*. This kind of crossing was carried out for two reasons: (1) to produce triple hybrids (*N. glauca* ×

N. Langsdorffii) × *N. Sanderae*, and at the same time (2) to produce some F_2 progenies from the F_1 hybrids. The former could be very easily

Text-fig. 18. Somatic plate from a trigenomal triple hybrid (*N. glauca* × *N. Langsdorffii*) × *N. Sanderae* with 30 chromosomes.



Text-fig. 19. (*N. glauca* × *N. Langsdorffii*) × *N. Sanderae* hybrid with 45 chromosomes. It had very coarse and thick leaves.

distinguished from the latter, since *N. Sanderae* used in these crosses had very large red flowers while both components of F_1 hybrids have greenish

yellow flower colour. Any triple hybrid would have large reddish flowers, while any F_2 progeny would not show any red. Flowers of twelve F_1 plants were self-pollinated and at the same time crossed with pollen from *N. Sanderae* over a period of two months. The pollinations were carried out soon after the flowers opened. The pollinations of certain flowers were sometimes repeated. The manipulation is very simple, and one worker can thus perform about ten times more crossings than in the case when castration and labelling of each flower is necessary. Exact counts of the pollinated flowers was not made, but, on the average, they can be estimated about 600–800 (i.e. about 182,000 ovules). From these crossings only a few capsules remained until their seeds matured, all the others dropping prematurely. A small amount of seeds was obtained from which hybrids were raised. This triple cross was carried out at various times and years, but seeds were obtained only in the way described above. It should be mentioned here that the pollen tubes of *N. Sanderae* easily reach the ovary of F_1 hybrids and almost every ovule receives a pollen tube. It is possible that *N. Sanderae* pollen tubes might make, as it were, a path through the hybrid styles and thus facilitate the pollen-tube growth of the F_1 large pollen. If the cross-pollinations were made with a small amount of *N. Sanderae* pollen grains, it seems very probable that from such combined pollinations one might produce amphidiploids. *N. Sanderae* pollen tubes passing through the F_1 styles might enter only into a part of the ovules, while the thicker pollen tubes of the F_1 hybrids passing perhaps easily through the style after *Sanderae* pollen tubes, might enter into the other part of the ovules, thus some of the sperms originating from dyads might meet egg cells originating from dyads, and after a fusion might give rise to amphidiploid embryos and plants. Similar crossings and similar results, as here theoretically outlined were obtained in working with another *Nicotiana* species hybrid, while from the triple cross (*N. glauca* × *N. Langsdorffii*) × *N. Sanderae* I obtained only triple hybrids, i.e. hybrids in which *N. Sanderae* has participated as a paternal plant. Triple hybrids (*N. glauca* × *N. Langsdorffii*) × *N. Sanderae* differed very greatly morphologically and physiologically. Even those that had thirty somatic chromosomes differed from each other in many respects. One of the causes for the great variability of the triple hybrids having a whole somatic set (21) from F_1 hybrid and the whole *Sanderae* genom is the occurrence of chromatid crossing-over in the F_1 hybrid, i.e. the same cause that conditioned the variability of the trigenomal hybrids (*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii* (30 chromosomes) obtained by a primary back-cross. The other cause is the heterozygosis of *N.*

Sanderae. The pollen-tube growth of this species is regulated by sterility factors which prevent inbreeding and favour cross-breeding as shown by East's school, therefore it is usually highly heterozygous.

I have studied cytologically twelve plants from the triple hybrids. Six of them had 30 somatic chromosomes (Text-fig. 18), two had 31 somatic chromosomes, one had 30 ± 1, one had 45, one had *ca.* 51, and one had about 23 somatic chromosomes. It is most probable that the plants with 30 somatic chromosomes originated from egg cells of F_1 hybrids having the somatic chromosome numbers (21). Such cells are formed, as mentioned above, following non-occurrence of the first meiosis. Plants with 31 chromosomes have originated from egg cells having 22 chromosomes, i.e. one more than the somatic chromosome number. Such egg cells might originate from restitution nuclei formed during the first meiosis and non-disjunction involving one chromosome during the second division, or from such restitution nuclei in which one univalent has divided during the first meiotic division. Both alternatives are probable; the first one, however, occurs more frequently.

In the same way have originated the plants with 31 somatic chromosomes of the primary back-cross (*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii*.

The triple hybrid with 45 somatic chromosomes has originated from an egg cell with 36 chromosomes. It is difficult to suggest just what kind of abnormalities in the meiosis have yielded such an egg cell, because there are very many possible ways.

The triple hybrid with about 51 somatic chromosomes has originated from an egg cell having 42 somatic chromosomes, i.e. the doubled somatic chromosome number of the F_1 hybrid. Such an egg cell might be formed when both meiotic divisions failed to occur (monad type). This phenomenon was observed during meiosis in the pollen mother cells.

The appearance of the plant with *ca.* 51 chromosomes suggests strongly that the amphidiploid plant with 42 somatic chromosomes which originated in crossing F_1 (*N. glauca* × *N. Langsdorffii*) with pollen of *N. Langsdorffii* resulted from parthenogenetic development of an egg cell with 42 somatic chromosomes. The origin of this egg cell is probably the same as that which gave rise to the triple hybrid with *ca.* 51 chromosomes (di-*glauca*—di-*Langsdorffii*—mono-*Sanderae*).

The meiosis of triple hybrids with 30 somatic chromosomes was very similar to that in the back-crosses (*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii* with 30 chromosomes. It should be mentioned here that the *N. Langsdorffii* ($n = 9$) genom is homologous with the *N. Sanderae* ($n = 9$)

genom. In most of the *N. Langsdorffii* × *N. Sanderae* F_1 hybrids, the chromosomes conjugate normally as in the pure species. I have only once found a structural hybrid *N. Langsdorffii* × *N. Sanderae*. Triple hybrids (*N. glauca* × *N. Langsdorffii*) × *N. Sanderae* were highly sterile. Only a very small amount of seeds was obtained from them by selfing.

The triple hybrid with 45 somatic chromosomes had very irregular meiosis, and was self-sterile.

The triple hybrid with *ca.* 51 chromosomes had also irregular meiosis. It often formed polyvalent chromosomes. In several cases a pentavalent group was found; most frequently, however, bivalents and trivalents were formed. It also formed about 7–10 univalents. This plant formed a large amount of very unequal viable pollen grains (80–92 %), but it was highly sterile, setting rarely only a few seeds per capsule. It was very robust with coarse, thick leaves, i.e. characters typical for polyploid plants (cf. Kostoff, 1938*k*).

The progeny of one triple hybrid with 30 chromosomes were bred through for three generations and then crossed again to *N. Sanderae*. These hybrids will be described elsewhere.

Allopolyploids

Crossings between *N. glauca* and *N. Langsdorffii* yielded the following types of allopolyploids:

(1) Plants with one genom of *N. glauca* and two genoms *N. Langsdorffii* (mono-*glauca*—di-*Langsdorffii*). They were obtained on crossing F_1 hybrids to *N. Langsdorffii*.

(2) Plant with three genoms of *N. glauca* and four genoms of *N. Langsdorffii* (tri-*glauca*—tetra-*Langsdorffii*). It was obtained on crossing a trigenomal back-cross (*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii* with *N. glauca*.

(3) Plants with one genom of *N. glauca*, one of *N. Langsdorffii* and one of *N. Sanderae* (mono-*glauca*—mono-*Langsdorffii*—mono-*Sanderae*). They were produced by crossing F_1 *N. glauca* × *N. Langsdorffii* with *N. Sanderae*.

(4) Plant with two *N. glauca*, two *N. Langsdorffii* and one *N. Sanderae* genom. (di-*glauca*—di-*Langsdorffii*—mono-*Sanderae*). It appeared from the cross F_1 (*N. glauca* × *N. Langsdorffii*) × *N. Sanderae*.

(5) Plants with two *N. glauca* and two *N. Langsdorffii* genoms, i.e. amphidiploids (di-*glauca*—di-*Langsdorffii*). They were obtained in the following ways: (*a*) On crossing F_1 hybrid with pollen of *N. Langsdorffii*,

(*b*) on crossing trigenomal hybrid (*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii* with *N. glauca*, and (*c*) on crossing *N. glauca* with pollen of trigenomal hybrid (*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii*. (The last died before reaching maturity.)

The amphidiploid *N. glauca—N. Langsdorffii* obtained parthenogenetically on crossing F_1 hybrid *N. glauca* × *N. Langsdorffii* with *N. Langsdorffii* pollen is the most interesting allopolyploid form. It was studied through six generations. Its behaviour throws light upon the behaviour of species originating in nature by allopolyploidy, therefore it will be described fully.

(*a*) *Morphology of the amphidiploid* N. glauca × N. Langsdorffii (N. Vavilovii). Morphological appearance of the amphidiploid hybrid, that originated parthenogenically was very much like the F_1 hybrids *N. glauca—N. Langsdorffii*. It had rather coarser and broader leaves, somewhat larger flowers and in all respects was more robust than the F_1 hybrid. The amphidiploid plant formed tumours at the end of its first florescence period. Amphidiploids obtained by gradual accumulation of genoms as described above differed morphologically in some respect from each other and from the F_1 hybrids.

In order to avoid repetition of long descriptions in defining the allopolyploid forms, I shall here call the amphidiploid *N. glauca—N. Langsdorffii*, that originated parthenogenetically, and its progenies as *N. Vavilovii*.[1] The necessity for a special name for the forms obtained from this amphidiploid will be seen after the description of its progenies.

(*b*) *Cytology of the parthenogenetically originated amphidiploid* (N. Vavilovii). In the root tips of the first plant I counted 42 chromosomes. About the same chromosome number was counted in the tumours, which it formed like the F_1 hybrids.

In studying the meiosis in the original amphidiploid I found quite often the formation of multivalents and the appearance of univalents. In studying 42 P.M.C. during the first meiosis I found 15 metaphases without multivalents, 16 with one multivalent, 8 with two multivalent, and 3 with more than two multivalent chromosome groups. The multivalents were trivalents and quadrivalents. Trivalent groups were usually accompanied by univalents. Twenty-one P.M.C. had at least one univalent out of 42 studied, i.e. 50 %. Some P.M.C. had two or more univalents. Trivalents

[1] N. I. Vavilov, Director of the Institute of Plant Industry of U.S.S.R. under whose guidance about 300,000 forms of cultivated plants and their wild-growing relatives were collected, studied and classified from an agricultural, physiological, geneecological and biochemical point of views.

were always in chain, while quadrivalents appeared in rings as well as in chains.

The appearance of multivalents and univalents led to unequal chromosome distributions to the poles. The equal chromosome distributions were detected by counting the second metaphases. In counting 52 second metaphases I found in 18 cells plates with 21 chromosomes, while the other 31 cells had plates with more or with less than 21 chromosomes. In studying two preparations I also found several second metaphase plates with 24 chromosomes and one with 25 chromosomes.

Multivalent chromosome groups obviously result from chiasma formation (crossing-over) following auto- and allosyndetic chromosome association of *N. glauca* and *N. Langsdorffii* chromosomes. The segments of *N. glauca* and *N. Langsdorffii* chromosomes which synapsed in the F_1 hybrids obviously synapse sometimes in the amphidiploid too, cross-over, and the chiasmata, thus formed, hold them until late metaphase.

Crossing-over that takes place in allosyndetically synapsed segments, leads to formation of genetically unequal gametes. In other words, multivalent formation in the amphidiploid is the signal: (1) for the occurrence of allosyndesis parallel with autosyndesis in the amphidiploid, and (2) for the formation of genetically unequal gametes: (*a*) in respect of a series of genes in the gametes having 21 chromosomes, and (*b*) in respect of the chromosome number.

These phenomena condition the inconstancy of the amphidiploid, its "segregations" and the origin of numerous forms that were shortly called *N. Vavilovii*.

(*c*) *Fertility of the amphidiploid.* The process of meiotic division is a reason for expecting the amphidiploid to form a certain percentage of non-viable gametes and not to be fully fertile. In studying the viability of the pollen in acetocarmine preparations I found that the original amphidiploid had about 51 % of viable pollen. The viable ones were not quite uniform in size, some being rather larger, others smaller. It should be mentioned, however, that both types germinated on the stigmas of *N. Vavilovii* as well as on *N. tabacum* stigmas.

The first five flowers were not artificially self-pollinated. Three of them dropped without setting seeds, while the other two formed capsules with a small amount of seeds (22 and 36 seeds per capsule). The next flowers, formed by the amphidiploid, were pollinated with its own pollen. Each capsule so produced remained on the plant until the full maturity of the seeds. Those capsules that were not collected, when they became brown, burst like the capsules of *N. Langsdorffii*. In ten capsules

obtained after self-pollination of the flowers I found altogether 480 large, well-developed seeds, i.e. on the average 48 seeds per capsule. Among these seeds, numerous small shrunken ovules were found.

In order to give an idea of the approximate degree of fertility of the first original amphidiploid I shall mention here that *N. glauca* forms on the average 804·8 seeds per capsule, *N. Langsdorffii* about 198 seeds per capsule, and the hybrid had about 260 ovules per capsule. The average number of seeds per capsule for *N. glauca* and *N. Langsdorffii* were obtained in counting the seeds from capsules of several plants at various seasons of the years when grown in the greenhouse (*N. glauca*: 778, 811, 971, 1029, 1037, 1112, 690, 659, 248, 715; *N. Langsdorffii*: 135, 153, 250, 223, 192, 208, 185, 188, 268, 235, 131).

The data recorded above show that the original amphidiploid plant had a significantly reduced fertility.

PROGENIES OF THE PARTHENOGENETICALLY PRODUCED AMPHIDIPLOID *N. GLAUCA*—*N. LANGSDORFFII*

In studying the meiosis of the original amphidiploid I noticed that the plant did not form gametes all alike. Hence the offspring should differ in many respects—cytogenetically, morphologically, physiologically, and biochemically.

(*a*) *Morphology of the progenies*

The first impression given by the second generation, as well as by some families of the third and fourth, is the lack of uniformity. Although the majority of the plants resembled each other and were very much like the first one produced parthenogenetically, there is a variable proportion of plants in almost every large family differing greatly from the original type in respect of the size and shape of the leaves and flowers, habit of growth, vegetation period, etc.

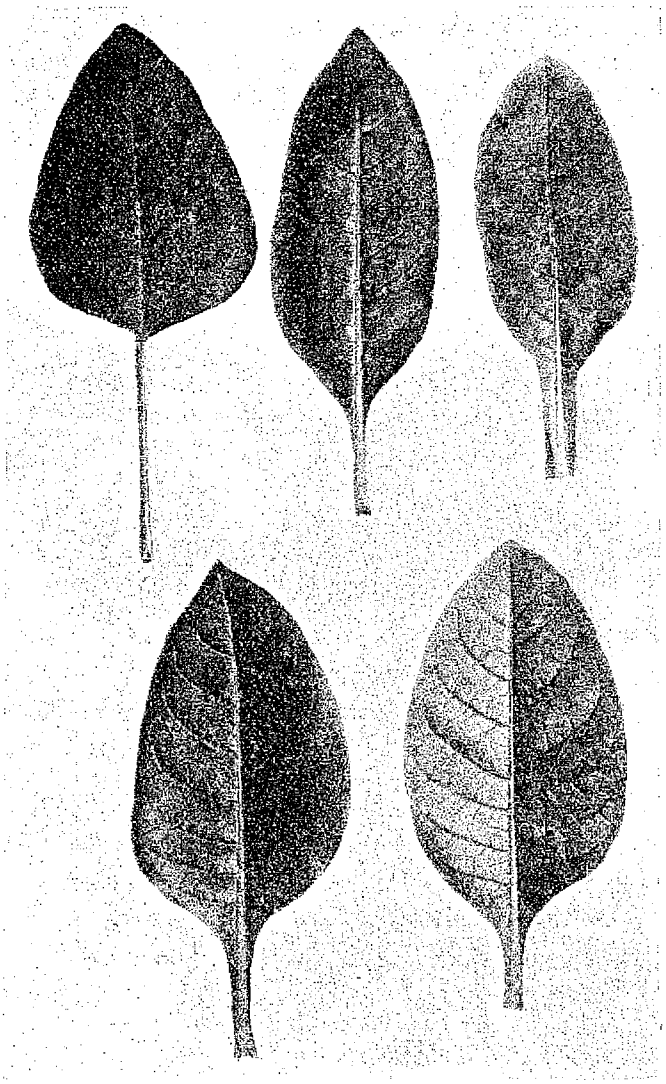
(1) *Second amphidiploid generation.*

In the second amphidiploid generation 32 % of the plants represented deviations from the original type.

Types of deviations. (*a*) *Leaves.* Plants occurred with leaves elongated in various degrees. Some had oval, others elliptical leaves; several had spatulate, and one had almost cordate leaves. The forms of the apex also varied greatly. There were plants with obtuse, acute, acuminate and even cuspidate apices.

(*b*) *Flowers.* The plants deviating from the original type had flowers that differed in respect to corolla tube length, corolla tube breadth, the

opening of the corolla, the position of the stigma in respect to the position of the anthers, and in the colour of the pollen. Some plants had much



Text-fig. 20. Leaves. First row: Left *N. glauca*, right *N. Langsdorffii*, in the middle F_1 hybrid. Second row: leaves of the allopolyploid *N. glauca—N. Langsdorffii* hybrid. Note the breadth of the leaves in respect to that of F_1 hybrid.

longer flowers, others much shorter than the original amphidiploid. Several plants had broader corollas than those of *N. Langsdorffii* and of

the original amphidiploid. The stigmas projected up to 3 mm. above the anthers in several segregates (6 %); in others the stigmas were 3 mm. below the anthers, and all transitional forms occurred. *N. Langsdorffii*, F_1 hybrids and the original amphidiploid had bluish violet pollen, while *N. glauca* had white pollen. I found several plants in F_2 amphidiploid generation with white pollen.

(c) *Habit of growth and vegetation period.* Some plants of the second amphidiploid generation grew with a main stem and began to form side branches at the end of the first flowering period, others began to form side branches before the differentiation of the first floral buds. Transitional forms also appeared. Some plants were dwarfs, others were much larger than the largest F_1 hybrids. Some plants began to flower at the same time as F_1 hybrids, while others were about six months later. The majority of the plants flowered between these two extreme types but closer to the earlier one. The types that were in appearance like the original amphidiploid flowered somewhat later than the F_1 hybrids. There were also plants with deformed leaves and flowers.

The original amphidiploid as well as all of its progenies formed non-parasitic tumours. Some plants formed tumours very abundantly with intensive necrosis and died before the differentiation of floral buds.

(2) *Third and further generations.*

In the third as well as in further generations families were grown from various F_2 types. Some appeared to be highly constant, giving about 2–5 % divergent types, others gave about 55 %, while in the majority of the F_3 families I found between 10 and 20 % of divergent types. The divergency is meant from the parental F_2 plant and prevailing F_3 type. In F_4 and F_5 numerous divergent types showed high constancy giving 1–2 % of new segregates. There were, however, plants that gave a large number of new segregates in F_4, F_5 and F_6. Three morphologically different families consisting of 130, 135 and 140 plants in F_6 did not give visibly divergent types while all the others, grown, gave various percentages of segregates. Partially fertile segregates usually gave many more divergent types than those that gave larger amount of seeds.

The types segregated in F_3, F_4, F_5 and F_6 involved all characters. The types obtained in F_2 were segregated anew in the subsequent generations with various gradations. Various types of leaves were combined with all possible variations of flowers and habit of growth.

N. glauca is a perennial bush, while *N. Langsdorffii* is a herbaceous plant. In the F_4 and F_5 generations plants appeared like the one and the

TABLE V

Length and breadth of the corolla in mm. of various families derived from the amphidiploid.
(The plants have various chromosome numbers)

	Length of the corolla in mm.													Breadth of the opening of the corolla in mm.										
	23	24	25	26	27	28	29	30	31	32	33	34	35	36	8	9	10	11	12	13	14	15	16	17	18	*n*
Amphidiploid *N. glauca* × *N. Langsdorffii*:																										
Plant No. 0351 (11)	.	.	.	.	.	.	2	3	.	.	.	.	.	.	.	.	.	.	.	1	2	2	.	.	.	5
No. 0351 (21)	.	.	.	3	2	.	.	.	.	.	.	.	.	.	.	.	2	3	.	.	.	.	.	.	.	5
No. 0351 (25)	.	1	4	1	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	5	2	1	10
No. 0351 (26)	.	1	3	4	.	2	.	.	.	.	.	.	.	.	4	3	3	.	.	.	.	.	.	.	.	10
No. 0351 (27)	.	.	.	.	.	8	2	.	.	.	.	.	.	.	.	.	1	1	7	1	.	.	.	.	.	10
No. 0351 (28)	.	.	.	.	.	5	3	2	.	.	.	.	.	.	.	.	.	.	1	5	4	.	.	.	.	10
No. 0351 (29)	.	1	4	3	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	7	3	.	.	10
No. 0351 (30)	.	.	3	2	5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	1	3	4	.	.	10
No. 0351 (31)	.	.	.	.	2	5	2	1	.	.	.	.	.	.	.	.	4	2	3	1	.	.	.	.	.	10
Total for No. 0351	.	3	14	13	12	23	9	6	.	.	.	.	.	.	4	3	10	6	11	10	7	14	12	2	1	80
Plant No. 5015 (1)	.	.	.	.	.	2	3	.	.	.	.	.	.	.	.	.	.	.	1	2	1	1	.	.	.	5
No. 5015 (2)	.	.	.	.	.	.	.	.	.	.	3	2	.	.	.	.	.	.	.	3	2	.	.	.	.	5
No. 5015 (3)	.	.	.	.	.	.	.	.	.	3	2	.	.	.	.	.	.	.	2	2	.	.	1	.	.	5
No. 5015 (17)	.	.	.	.	.	.	.	.	.	.	1	4	.	.	.	.	.	.	.	3	2	.	.	.	.	5
No. 5015 (18)	.	.	.	.	.	.	.	2	1	1	1	.	.	.	.	.	.	.	1	1	3	.	.	.	.	5
No. 5015 (16)	.	.	.	.	.	.	.	1	1	1	2	.	.	.	.	.	.	.	.	4	1	.	.	.	.	5
No. 5015 (19)	.	.	.	.	.	.	.	.	.	3	2	.	.	.	.	.	.	1	1	2	1	.	.	.	.	5
No. 5015 (20)	.	.	.	.	.	.	.	.	.	5	.	.	.	.	.	.	.	.	.	.	2	.	1	2	.	5
No. 5015 (100)	.	.	.	.	.	.	1	.	.	2	2	.	.	.	.	.	.	.	.	.	.	2	2	.	1	5
No. 5015 (101)	.	.	.	.	.	.	.	.	.	5	.	.	.	.	.	.	.	.	2	1	1	1	.	.	.	5
No. 5015 (102)	.	.	.	.	.	.	.	.	.	1	.	2	2	.	.	.	.	.	.	4	1	.	.	.	.	5
No. 5015 (108)	.	.	.	.	.	.	.	.	2	2	1	.	.	.	.	.	.	.	.	.	1	4	.	.	.	5
No. 5015 (5)	.	.	.	.	.	.	.	.	2	5	.	.	.	.	.	.	.	.	1	1	1	2	2	.	.	7
Total for No. 5015	.	.	.	.	.	2	4	3	6	28	14	8	2	.	.	.	.	1	8	23	16	10	6	2	1	67

Plant No. 526 (1)	.	.	.	.	1	.	1	3	1		.	.	.	.	.	.	.	.	1	.	3	2	.	.	.	6
No. 526 (3)	.	.	.	.	.	2	1	2	1	.	.	.	.	.	.	.	.	1	.	3	2	1	.	.	.	6
No. 526 (4)	1	2	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	2	2	2	.	.	.	.	.	6
No. 526 (8)	.	.	.	.	2	4	.	.	.	.	.	.	.	.	.	.	.	.	2	.	4	.	.	.	.	6
No. 526 (9)	.	.	.	.	1	2	.	1	2	.	.	.	.	.	.	.	.	.	1	3	1	1	.	.	.	6
No. 526 (10)	.	.	.	2	.	4	1	.	.	.	.	.	.	.	.	.	1	2	1	1	2	.	.	.	.	7
Total for No. 526	1	2	2	3	4	12	3	6	4	.	.	.	.	.	.	.	1	5	7	8	12	4	.	.	.	37
Plant No. 527 (52)	.	.	.	.	.	.	.	.	3	4	1	.	.	.	.	.	.	1	1	3	3	.	.	.	.	8
No. 527 (2)	.	.	.	.	.	3	2	.	1	.	.	.	.	.	2	.	1	1	2	.	.	.	.	.	.	6
No. 527 (51)	.	.	.	.	1	1	1	2	.	1	.	.	.	.	.	.	.	1	3	2	.	.	.	.	.	6
No. 527 (50)	.	.	.	.	3	4	.	.	.	.	.	.	.	.	.	.	2	4	1	.	.	.	.	.	.	7
No. 527 (56)	.	1	.	1	2	1	.	1	.	.	.	.	.	.	.	.	.	.	2	3	1	.	.	.	.	6
No. 527 (4)	.	.	.	.	.	1	4	1	.	.	.	.	.	.	.	.	.	.	.	1	4	1	.	.	.	6
Total for No. 527	.	1	.	1	6	10	7	4	4	5	1	.	.	.	.	2	2	6	6	12	10	1	.	.	.	39
Plant No. 573 (1)	.	.	.	.	.	3	2	.	1	.	.	.	.	.	.	.	.	.	2	1	2	1	.	.	.	6
No. 573 (3)	.	.	.	.	.	2	1	2	1	.	.	.	.	.	.	.	.	.	2	2	1	1	.	.	.	6
No. 573 (54)	.	.	.	.	.	.	.	.	2	1	2	.	.	.	.	.	.	.	2	3	.	.	.	.	.	5
No. 573 (55)	.	.	.	.	.	.	.	.	1	2	3	.	.	.	.	.	.	5	1	.	.	.	.	.	.	6
No. 573 (2)	.	.	.	.	.	4	1	1	.	.	.	.	.	.	.	.	.	.	1	.	4	1	.	.	.	6
No. 573 (59)	.	.	1	2	1	3	.	.	.	.	.	.	.	.	.	.	.	.	1	.	3	2	.	1	.	7
No. 573 (60)	.	.	.	.	.	4	2	2	.	.	.	.	.	.	.	.	.	.	.	.	3	2	3	.	.	8
Total for No. 573	.	.	1	2	1	16	6	5	5	3	5	.	.	.	.	.	.	5	9	6	13	7	3	1	.	44
Total for the amphidiploid	1	6	17	19	23	63	29	24	19	36	20	8	2	.	4	5	13	23	41	59	58	36	21	5	2	267
F. glauca × *Langsdorffii*	.	.	2	42	87	79	31	9	.	.	.	.	.	.	.	.	18	88	96	48	.	.	.	.	.	250
N. glauca	.	.	.	.	.	.	2	7	18	3	.	.	.	.	12	15	3	.	.	.	.	.	.	.	.	30
N. Langsdorffii	.	5	15	7	.	.	.	.	.	.	.	.	.			.		4	21	5	.	.	.	.	.	30

other parental species, the majority being intermediate but fluctuating very greatly.

I shall recall here the transgressions observed in the progenies of the amphidiploid estimated in respect of the F_1 and the original amphidiploid: (1) larger leaves, (2) broader leaves, (3) great variations in the leaf apex, (4) transgressive variations in the shape of the leaves, (5) longer flowers, (6) broader and narrower corolla, (7) longer and shorter styles

TABLE VI

Leaf index length: breadth and petiole length of plants from various families derived from the amphidiploid N. glauca—N. Langsdorffii. (*The plants have various chromosome numbers*)

No. of the family and of the plant	Average leaf index length: breadth	Average length of the petioles in mm.
(1) 526— 1	1·4	30
(2) 526— 3	1·37	29
(3) 526— 4	2·2	20
(4) 526— 8	1·8	26
(5) 526— 9	1·5	30·3
(6) 526—10	1·5	31
(7) 527— 2	1·4	32
(8) 527— 4	1·6	22
(9) 527—50	1·4	16
(10) 527—51	1·8	21
(11) 527—52	1·4	22
(12) 527—26	1·54	20
(13) 528— 2	2·16	22
(14) 530— 1	1·7	21
(15) 573— 1	1·65	19
(16) 573— 2	1·7	23
(17) 573— 3	1·6	32·3
(18) 573—54	1·56	27·6
(19) 573—55	1·6	33
(20) 573—59	1·8	18
(21) 573—60	1·66	22
(22) 585—53	1·66	34
(23) 585—58	1·6	24
(24) 595— 2	1·65	28
(25) 595— 1	1·6	17
(26) 595—57	1·6	16·6
(27) 5015— 5	1·4	21

(the position of the style with respect to the position of the anthers), (8) white pollen, (9) longer and shorter vegetation period, (10) earlier and later formation of side branches, (11) herbaceous and bushy type of plants, (12) formation of much larger and much smaller non-parasitic tumours (Tables VI–VIII, Text-figs. 21–26).

Amphidiploid forms ($2n=42$) have on the average broader leaves (cf. Kostoff, 1938*k*) and larger flowers than the F_1 hybrids.

Tumour formation by the amphidiploid and all its progeny is a remarkable phenomenon. By doubling the chromosome number in F_1

TABLE VII

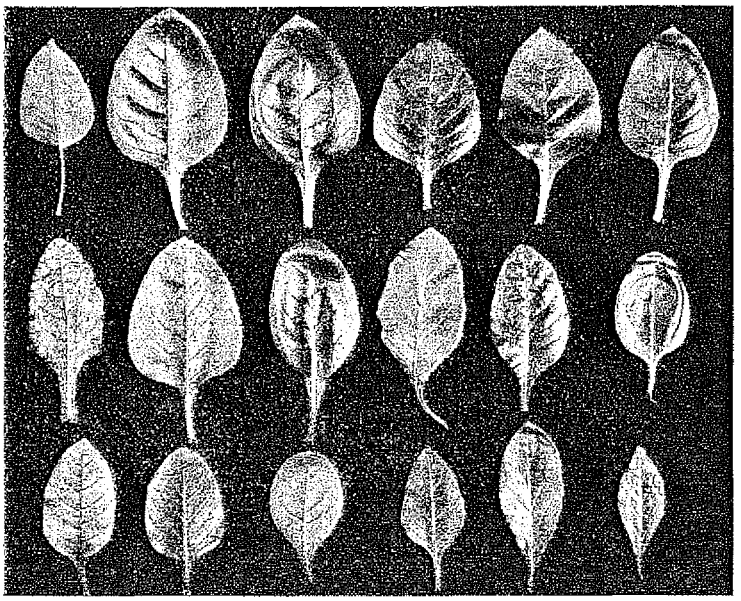
Leaf index length : breadth of F_1 hybrid and of the plants of a uniform family of the amphidiploid N. glauca—N. Langsdorffii ($2n = 42$)

Forms	Somatic chromosomes	Leaf index length:breadth 1·30–1·39	1·40–1·49	1·50–1·59	1·60–1·69	1·70–1·79	1·80–1·89	1·90–1·99	2·00–2·09	2·10–2·19	n	M	$M_1 - M_2$ $\pm m_{diff.}$
(1) F_1 hybrid	21	—	—	—	3	18	24	15	4	1	15	1·88	0·30 ±0·02
2) Amphidiploid	42	2	15	24	23	8	1	—	—	—	73	1·58	

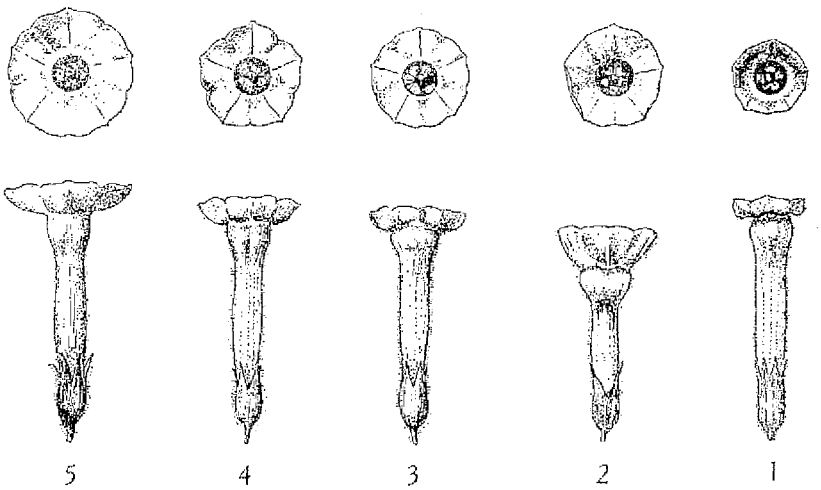
TABLE VIII

Plant size (height) at the end of the florescence period

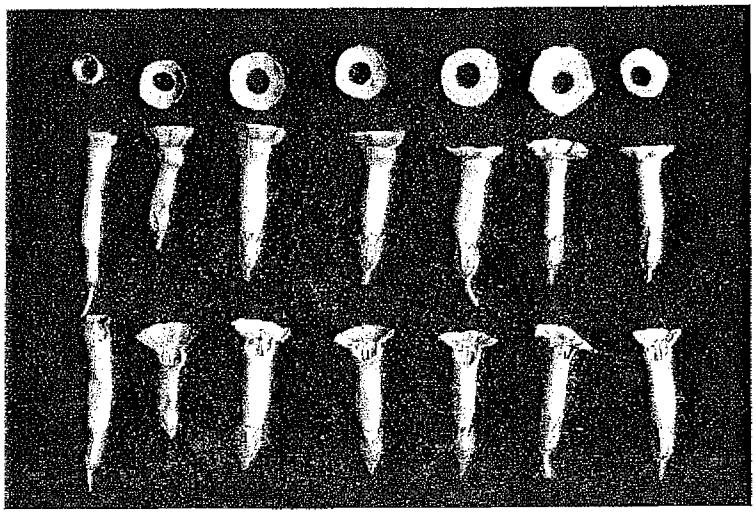
Plants	–50	51–60	61–70	71–80	81–90	91–100	101–110	111–120	121–130	131–140	141–150	151–160	n	M
F_1 *N. glauca* × *N. Langsdorffii*	—	—	—	8	10	6	1	—	—	—	—	—	25	85
Amphidiploids *N. glauca* × *N. Langsdorffii*:														
Family 5015	—	—	—	—	—	—	1	1	4	4	—	2	12	131
Family 351	1	—	—	3	5	1	5	1	—	—	—	—	16	89·5
Family 585	—	—	—	—	—	—	—	1	—	4	6	4	15	173
All three families of amphidiploids	1	—	—	3	5	1	6	3	4	8	6	6	43	119·7



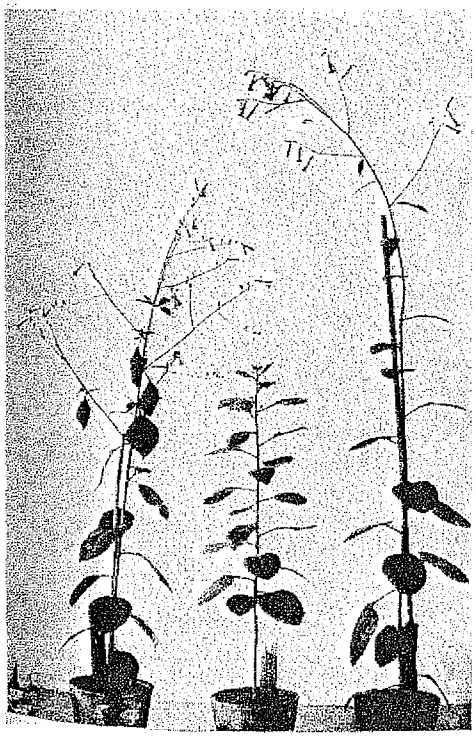
Text-fig. 21. Leaves. Left, first row, *N. glauca*; left, second row, *N. Langsdorffii*; left, third row, F_1 hybrid *N. glauca—N. Langsdorffii*. The other leaves are taken from various plants of the progeny of *N. glauca—N. Langsdorffii* amphidiploid. Amphidiploids had broad leaves. Some of the aberrants had narrow leaves.



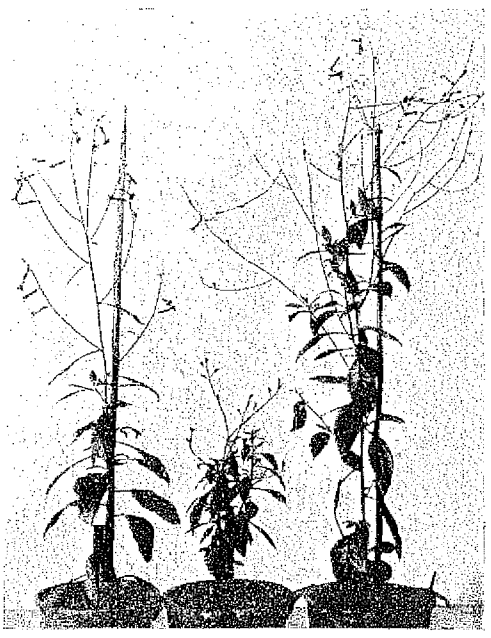
Text-fig. 22. Flowers (natural size). From right to the left: (1) *N. glauca*, (2) *N. Langsdorffii*, the others (3), (4) and (5) flowers from different plants of the progeny of an amphidiploid. Note the variability. (Drawn by N. Dogadkina.)



Text-fig. 23. Flowers. From left to the right, in both rows. First *N. glauca*, second *N. Langsdorffii*, the other flowers (3rd to 7th) from different plants of the progeny of *N. glauca—N. Langsdorffii* amphidiploid.



Text-fig. 24.



Text-fig. 25.

Text-figs. 24, 25. Sister plants raised from seeds obtained by self-pollination the amphidiploid *N. glauca—N. Langsdorffii*. The plants were grown at equal environmental conditions in the greenhouse.

N. glauca—N. Langsdorffii I obtained the plant *N. Vavilovii*, which has the new character—"formation of non-parasitic tumours"—that is not present in the parental species.

(b) Cytology of the progenies of the amphidiploid

Numerous plants of the second, third, fourth and some of the fifth and sixth generations were studied cytologically. Space does not allow me to give here a detailed description of the chromosome behaviour of

Text-fig. 26. Tumours formed by the progeny of the amphidiploid *N. glauca—N. Langsdorffii*. Left "aneuploid" plant with 43 somatic chromosomes, right amphidiploid ($2n=42$).

various plants in a large number of families of several generations, therefore I shall only call attention to the most important phenomena observed.

In studying the somatic chromosome number of a series of plants of various families the following chromosome numbers were found: 21, 23, 41, 42, 43, 44, 45, 46, 47, 48, 49, *ca.* 50, 51, 52 (Text-figs. 27–29). Plants with 21, 23 and 52 chromosomes were not found in the F_2 generation of the original amphidiploid. The majority of the plants studied had 42 chromosomes. Chromosome numbers 43, 44 and 48 occurred quite often. I shall give first the chromosome number of three F_3 families obtained from F_2 plants with 42, 43 and 44 somatic chromosomes as illustrating the karyotypic variability.

(1) Plants obtained by selfing an F_2 amphidiploid with 42 somatic

chromosomes had the following chromosome numbers: twelve had 42 chromosomes, five had 43, one had 41 and one had 45.

(2) Plants obtained by selfing the F_2 plant (hyper-amphidiploid) with 43 chromosomes, had the following chromosome numbers: six plants had 42 chromosomes, five had 43, six had 44, one had 44 (± 1), one had about 46, two had 48.

Text-fig. 27.

Text-fig. 28.

Text-fig. 29.

Text-fig. 27. Somatic plate from the plant given in Text-fig. 26, left ($2n=48$).

Text-fig. 28. Somatic plate from a plant of the progeny of *N. glauca*—*N. Langsdorffii* amphidiploid with 49 chromosomes.

Text-fig. 29. Somatic plate from a plant of the progeny of *N. glauca*—*N. Langsdorffii* amphidiploid with 23 somatic chromosomes.

(3) The following karyotypes were obtained by self-pollinating the F_2 plant with 44 chromosomes: two plants had 42 chromosomes, one had 43, seven had 44, two had 45, one had *ca.* 46, two had 45, one had about 50, and one had 49 (± 1).

These three families, as well as others that were cytologically analysed had a large number of plants, but all were not studied cytologically.

Here I may also give details for some F_4 plants. From one F_4 plant having 42 chromosomes, eleven offsprings had 42 chromosomes and one

had about 43. From an F_4 plant with 44 chromosomes, twelve plants had 44 chromosomes, three had 43, one had 42, two had 45 and one had about 48 ($\pm$ 1). Finally, in the progeny of an F_4 plant with 48 chromosomes, nine plants had 48 chromosomes, two had 47, one had 49 and two had about 50.

The examples given above show that plants with 42, 44 and 48 chromosomes tend to produce further plants with the same chromosome number. One F_3 plant, however, which had 44 chromosomes, gave rise to plants in the F_4 generation with the following chromosome numbers: eight had 42 chromosomes, three had 43, two had 44, two had 45, one had about 46, and one had 48–50. This plant was exceptional in giving rise to many plants with chromosome numbers other than its own.

In an F_3 plant with 42–43 somatic chromosomes I found that the long arm of the satellite chromosome of *N. glauca* was significantly reduced (Text-fig. 33). In an F_4 plant with 44 chromosomes the satellite chromosome was changed into a long chromosome with almost median constriction (Text-fig. 33). *These observations show that parallel with the numerical changes of the chromosomes, fundamental chromosome reconstructions take place in the amphidiploids originating from F_1 hybrids with partial allosyndesis.*

The most important statements that can be made on the basis of the above studies are as follows:

(1) The chromosome number of the plant obtained by self-pollinating the original amphidiploid in F_2, F_3, F_4, F_5 and F_6 generations is not necessarily 42. In other words *amphidiploid* N. glauca × N. Langsdorffii *was not constant morphologically and cytologically. It gave rise to plants with various karyotypes, structurally and numerically.*

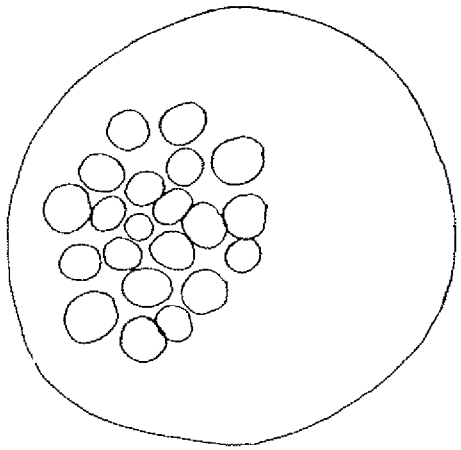
(2) Some of the plants with new chromosome numbers (44, 48, etc.) tended to reproduce further plants with the same chromosome numbers, but there were also such plants that gave predominantly offspring with new karyotypes.

The statements made in (1) and (2) are of great phylogenetic significance, since allopolyploidy of this kind, which is at the beginning an *euploid chromosome alteration, leads further to aneuploidy.* But even the euploid plants having 42 chromosomes were not equal morphologically and biochemically (Tables XVI, XVII). The great phylogenetic significance of these statements will be considered later. Divergency of the various forms of the amphidiploid with 42 chromosomes is gradually increased in subsequent generations, the frequency of appearance of new types is, however, decreased. The cause of this phenomenon is the occur-

rence of crossing-over between *N. glauca* and *N. Langsdorffii* chromosomes in the amphidiploids, i.e. the same cause which conditioned the formation of unequal gametes in the original amphidiploid plant.

Aneuploidy augments the numbers of new forms in the progenies of the amphidiploid *N. glauca—N. Langsdorffii* and leads to more striking divergency in the new forms.

Meiosis in the forms with 42 chromosomes of the second, third, fourth and fifth generations resembles the meiosis in the original amphidiploid with gradual decrease of the abnormalities (Text-figs. 30–32). One plant of the second generation formed definitely more multivalents than the

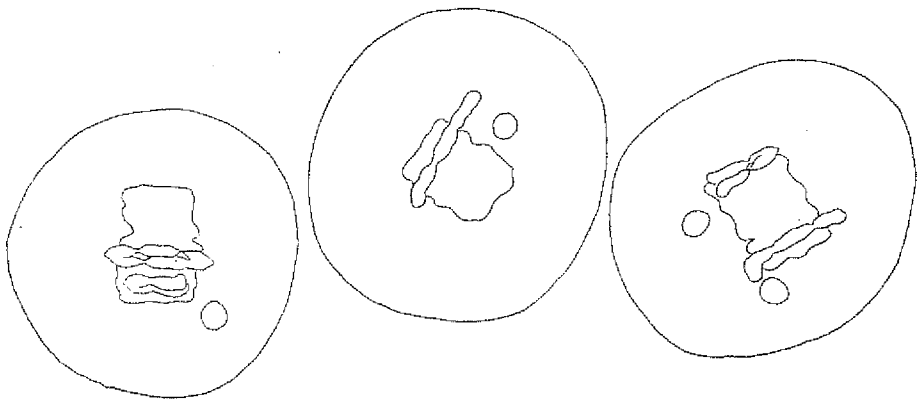


Text-fig. 30. Polar view of a metaphase plate from a pollen mother cell of the amphidiploid *N. glauca—N. Langsdorffii* ($n=21$).

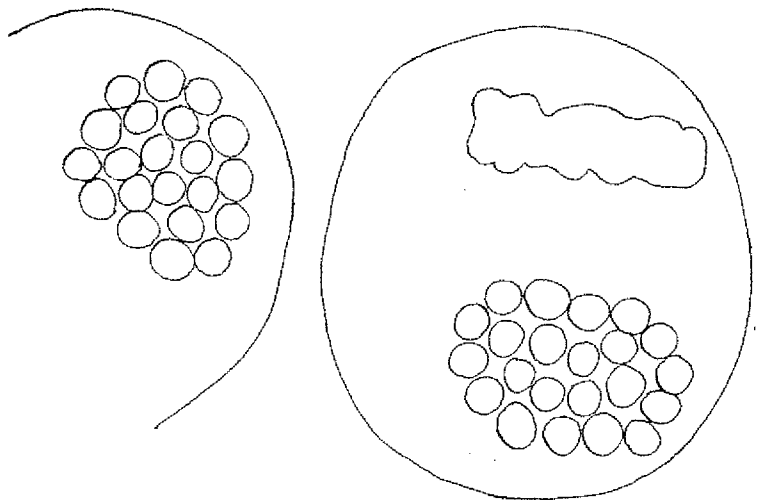
original amphidiploid. In two F_5 plants with 42 chromosomes, I very rarely found multivalent and univalent chromosomes. These observations indicate that a gradual differentiation of the chromosomes might take place which might further increase relatively the constancy of the plants with 42 chromosomes.

A comparison of the meiotic phenomena for two F_2, F_4 and F_6 plants, as given in Tables IX and X, shows definitely that the meiotic irregularities decrease with the increase of the number of generations. The plants of the second generations formed many more multivalents and univalents than those of the fourth generation, while those of the sixth generation had almost normal meiosis. The percentage of the abortive pollen decreases with the decrease of the irregularities in meiosis. Special attention should be called to the viability of the pollen in some plants of F_6 generation. Two amphidiploid plants studied had about 99·4% viable

pollen, while the parental plants, growing in the same conditions in the greenhouse, had the following percentages of abortive pollen grains: (1) *N. glauca*: plant 1, 98·2 %; plant 2, 97·3 %; plant 3, 93·4 %; *N. Langsdorffii*: plant 1, 99·0 %; plant 2, 96·8 %; plant 3, 95·1 %.

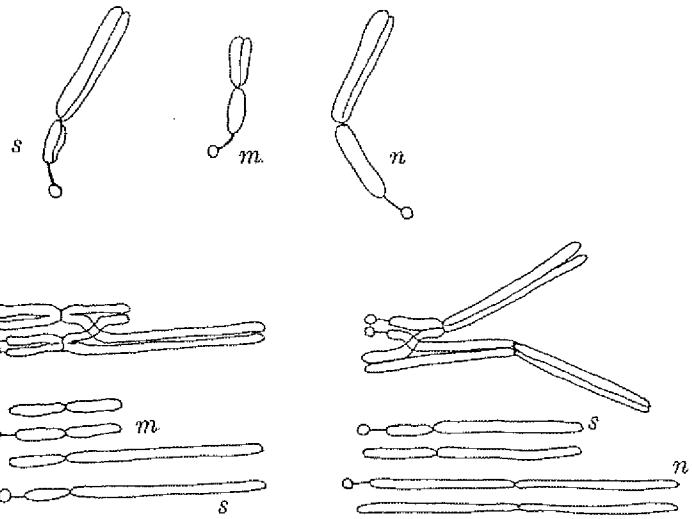


Text-fig. 31. P.M.C.'s of the amphidiploid *N. glauca—N. Langsdorffii* during the first meiotic metaphase (side view) with multivalents (trivalents and quadrivalents) and with univalents.

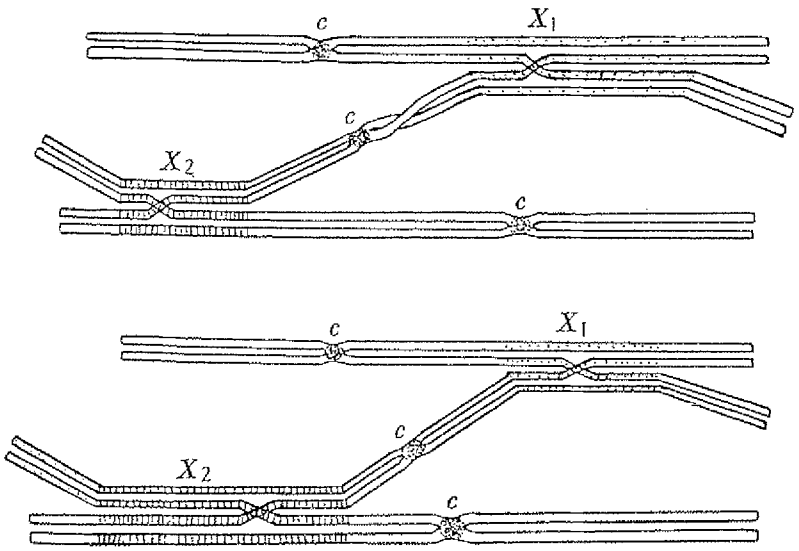


Text-fig. 32. Second metaphase of the amphidiploid *N. glauca—N. Langsdorffii* with 21 and 22 chromosomes.

The frequency of formation of second metaphase with normal chromosome number (21) increases with the increase of the number of generations, F_4 amphidiploid having a smaller percentage of second metaphases with 21 chromosomes than the F_6 and a larger percentage of



Text-fig. 33. First row: *s*, normale satellite chromosome; *m*, satellite chromosome with shortened long arm; *n*, satellite chromosome with elongated short arm. Down: left, diagram showing the mode of origin of chromosome *m*; right, diagram showing the mode of origin of the chromosome *n*.



Text-fig. 34. Diagram showing the chiasma formation in partially homologous trivalent chromosomes. *X*, place of chiasma formation (crossing-over); *c*, centromeres. Above: one and the same chromatid of the middle chromosome participates in both chiasmata X_1 and X_2, new chromosome thus formed will have changes ("translocations") at both ends. Three chromatids out of six will be changed. Down: one chromatid of the middle chromosome takes part in one chiasma (X_1) while the other chromatid—in the other (X_2). Four chromatids out of six (two-thirds) will be changed.

such metaphases than the F_2 amphidiploids (Table X). This influences greatly the gradual increase of the viable pollen with the increase of the number of generations. It also increases the fertility of the plants in the subsequent generations (Diagram, Text-fig. 35), thus gradually supplying better material from the evolutionary point of view for persistence in the struggle for survival. Plants with larger chromosome numbers (43, 44, 45, 46, 47, 48, etc.) have more frequently, and many more, multivalent and univalent chromosomes than the straight amphidiploids ($2n=42$). In the plants with 44 and 48 chromosomes, that were studied more extensively, trivalent, quadrivalent, and even pentavalent groups were found. In one plant with 48 chromosomes I even found several P.M.C.'s with hexavalent chromosome groups. Multivalency is a very important phenomenon, which leads to further cytogenetic divergency of the forms derived from the amphidiploid *N. glauca—N. Langsdorffii.*

In studying more thoroughly the meiosis of an amphidiploid of the F_5 generation (Pl. IV, figs. 1–11 and 17) having 42 chromosomes, I observed the following phenomena: (1) The plant usually formed 21 bivalents, occasionally one, two and rarely three univalents being found. Cells with one and three univalents had one trivalent. Quadrivalents were found very rarely. In studying about 50–60 P.M.C. I could find only one that had a quadrivalent chromosome group. Cells with two univalents occurred more often than those with one and three. (2) Heteromorphic bivalents were formed like those in F_1 hybrids, one component being considerably longer than the other. It cannot be affirmed with certainty that one of the chromosomes of the heteromorphic bivalents belongs to *N. glauca,* the other to *N. Langsdorffii,* because exchange of chromosome segments has proceeded in the F_1 hybrid as well as in the ancestors of the amphidiploid (F_1-F_4 amphidiploids), so that the shorter and/or the longer ones might be chromosomes with rearrangements following interspecific hybridization. (3) In some (1) anaphases (side view) one or two bivalents, having usually two (sometimes one) chiasmata, had delayed terminalization. They remained on the spindle when the other bivalents had already separated, and their components (the chromosomes) already occupied a polar position. It is very probable that delay of terminalization is conditioned by partial homology of the component chromosomes, which might have a secondary origin, resulting from chromosome rearrangements in the previous amphidiploid generations or in F_1 hybrid. (4) Some of the chromosome pairs of the amphidiploid had absorbed more dye during the first meiotic metaphase, others somewhat less, so that some of the pairs appeared black while the others were lighter, though somewhat darker

than the cytoplasm. The number of the lighter pairs was not strictly constant. It would not be quite correct if I divided the pairs into two groups: dark and light, because the latter had absorbed different quantities of dye and were not equally "light". The lighter pairs occupied various positions. They could be found in the middle of the metaphase plate, nearer to the object glass, or nearer to the cover glass, when one studies metaphase plates in side (equatorial) view. If they occupied constantly the position in the upper part of the equatorial plate (i.e. nearer to the cover glass) one would suppose, that, being almost on the surface, they were first distained. Their variable positions in the metaphase plate do not allow us to make such an assumption. It is true, that a large number of P.M.C.'s which have been slightly or severely injured by the microtome had "lighter" pairs, but there were also P.M.C.'s that appeared uninjured and had "lighter" pairs. In several instances I found that one of the chromosomes of a pair was darker than the other when the terminalization was to its end. This kind of differential staining is tempting me to assume that the lighter pairs have more *N. Langsdorffii* chromatine than the darker ones, since the chromosomes of pure *N. glauca* species absorb more dye and retain it longer during the differentiation, while *N. Langsdorffii* chromosomes can be more rapidly distained when the preparations are prepared by the same method. In addition to this I shall recall the observations made ten years ago on the meiosis of F_1 *N. glauca*—*N. Langsdorffii* hybrids when some of the univalents and one of the partners of the bivalents stained somewhat darker than the others. But in this case the number of the lighter and the darker also varied, and did not quite correspond to the parental chromosome numbers 12 and 9. There are two more objections against an assumption that the lighter chromosome pairs in the amphidiploid have chiefly *N. Langsdorffii* chromatin, and the darker ones—*N. glauca* chromatin. They are: (1) Crossing-over that proceeded in the F_1 hybrids and in some earlier amphidiploid generations ($F_1 - F_4$) leads to exchange of parts, consequently a large number of the chromosomes should consist of *N. glauca* and *N. Langsdorffii* segments. (2) On the basis of the chromosome theory of inheritance, the genes are linearly arranged in the chromosomes. If a *N. glauca* chromosome *a b c d e f g h* has a homologous segment (*e f g h*) with a *N. Langsdorffii* chromosome *m p q e f g h* one would expect, that, biochemically, these two chromosomes would react differently along their length as they do when their chromosome structure is revealed (Wenrich, 1916; Kostoff, 1938), reminding one of the reactions of the chromosomes in *Drosophila* salivary glands (Kostoff, 1930*d*; Painter, 1933) to various dyes.

This objection would be valid if the genes were arranged at equal distances in the chromosomes. More recent cytogenetic investigations in *Drosophila* and in some plants has shown, however, that almost every chromosome has heterochromatic, genetically inert regions where active genes are lacking, or are not as close together as in other regions (cf. Muller & Gershenson, 1935; Kostoff, 1938*g*, *h*). Heterochromatic regions absorb more dye than the euchromatic ones. The chromomere substances stained darker than the interchromomeric substances; the former were more closely packed in the heterochromatic regions. Since *N. glauca* chromosomes stained darker than those of *N. Langsdorffii* and did not distain as rapidly as *N. Langsdorffii* chromosomes, it is very probable that they have more heterochromatic substance (larger regions) in the chromosomes than the *N. Langsdorffii* chromosomes. This postulate is also supported by the fact that *N. glauca* is one of *Nicotiana* species having the longest chromosomes.

In plants as well as in animals the heterochromatic regions are usually found near the centromeres (i.e. at the proximal ends) and at the distal ends (Painter & Stone, 1935; Heitz, 1935; Prokofieva, 1935–7; Frolova, 1936, 1937; Kostoff, 1938*b*, *h*).

In studying more thoroughly the bivalents during the first metaphase in the amphidiploid *N. glauca—N. Langsdorffii*, I succeeded, after proper staining and distaining, in revealing heterochromatic regions in the proximal segments turned toward the poles (Text-figs. 1–4, 6–8, 11), and in some pairs at the very distal ends.

More recent investigations upon the chromosome behaviour in *Drosophila* salivary glands has shown that heterochromatic regions of non-homologous chromosomes conjugate together, usually forming a common chromocenter (Painter & Stone, 1935; Heitz, 1935; Prokofieva, 1935–37; Frolova, 1936). Similar phenomena have also been observed in plants. Kihara & Katayama (1933) and Chizaki (1935) found that during the first meiosis in haploid *Triticum monococcum* ($n=7$) usually an end-to-end chromosome association takes place. My investigations, directed toward the revealing of heterochromatin in *Triticum monococcum* showed that the distal ends of the majority of chromosomes of this species have heterochromatic regions, which are obviously responsible for the end-to-end associations in the haploids.

Considering these data it seems to me very probable, that some of the bivalents in F_1 *N. glauca—N. Langsdorffii* and perhaps some of the multivalents in the allopolyploid forms of the first and second generations

TABLE IX

Bivalent, multivalent, and univalent chromosomes observed during the first meiotic metaphase in the amphidiploids N. glauca—N. Langsdorffii ($2n=42$)

	Types of chromosome associations													
Plants	21 B	20 B 2 U	19 B 1 T 1 U	19 B 4 U	18 B 1 T 3 U	18 B 6 U	19 B 1 Q	18 B 1 Q 2 U	17 B 1 Q 1 T 1 U	17 B 2 Q	17 B 2 T 2 U	Total cells	% of cells with 21 B	% of viable pollen
Plant 1 of F_2	9	4	2	2	4	1	3	2	1	2	—	30	30	58
Plant 2 of F_2	11	3	3	1	3	—	2	3	1	2	1	30	37	60
Total for F_2 plants	20	7	5	3	7	1	5	5	2	4	1	60	33	59
Plant 1 of F_4	21	2	3	—	—	—	3	—	—	—	1	30	70	93
Plant 2 of F_4	22	2	2	1	—	—	2	—	—	1	—	30	73	95
Total for F_4 plants	43	4	5	1	—	—	5	—	—	1	1	60	72	94
Plant 1 of F_6	29	—	—	—	—	—	1	—	—	—	—	30	97	99·5
Plant 2 of F_6	30	—	—	—	—	—	—	—	—	—	—	30	100	99·4
Total for F_6 plants	59	—	—	—	—	—	1	—	—	—	—	60	98	99·45

B = bivalent, U = univalent, T = trivalent, Q = quadrivalent.

were results of chromosome associations between the heterochromatic regions of the non-homologous chromosomes.

In describing the cytological behaviour of the allopolyploids *N. glauca —N. Langsdorffii*, the origin and behaviour of two plants should be specially considered. One plant had 23 (Text-fig. 29), the other 21 somatic chromosomes. The former had probably a parthenogenetic origin from egg cell with 23 chromosomes. The other plant was obtained when the amphidiploids *N. glauca—N. Langsdorffii* were crossed with the

TABLE X

The chromosome number of single metaphase plates during the second meiotic division in the amphidiploids N. glauca—N. Langsdorffii ($2n=42$)

	Chromosome numbers of second metaphases											
Plants	17 and less	18	19	20	21	22	23	24	25 and more	Total cells	% of normal plates	% of viable pollen
Plant 1 of F_2	—	2	1	6	12	4	2	3	1	31	39	58
Plant 2 of F_2	2	1	2	3	15	6	1	—	2	32	46	60
Total for F_2	2	3	3	9	27	10	3	3	3	63	43	59
Plant 1 of F_4	—	—	—	2	26	1	—	1	—	30	87	93
Plant 2 of F_4	—	—	—	—	28	2	—	—	—	30	93	95
Total for F_4	—	—	—	2	54	3	—	1	—	60	90	94
Plant 1 of F_6	—	—	—	—	30	—	—	—	—	30	100	99·5
Plant 2 of F_6	—	—	—	—	32	—	—	—	—	32	100	99·4
Total for F_6	—	—	—	—	62	—	—	—	—	62	100	99·45

Remark: I counted both plates in plant 1 of F_2 generation in two P.M.C.'s, one having 19 the other 23. One of these cells was included in column 19, the other in 23. In plant 2 of F_2 generation one 20:22 cell, found, was included in column 22, while one 19:23, found, was included in column 19. Cells with 21 : 21 were counted as one having 21 chromosomes and were included in column 21.

amphidiploids *N. rustica—N. tabacum*. In pollinating 72 flowers of *N. glauca—N. Langsdorffii* with pollen from *N. rustica—N. tabacum* amphidiploid, twelve capsules were produced, all of them having shrunken seeds. From these seeds only one plant was grown and it was morphologically like *N. glauca—N. Langsdorffii* hybrids and had 21 somatic chromosomes. Theoretically, this plant should be like F_1 hybrids (*N. glauca—N. Langsdorffii*), in reality it was not identical with them, obviously, because the amphidiploids were not constant and formed unequal gametes. It had irregular meiosis, like the F_1 hybrids, forming 5–9 bivalents, some of them being heteromorphic.

Size of the cells in the allopolyploid forms

In studying the size of the cells in the polyploid forms the less variable ones were measured, namely: stomata guard cells, pollen mother cells, and pollen grains. It was found that each additional genom leads

TABLE XI

Length of the stomata cells in microns

Forms	Somatic chromosomes	Length in microns											
		22·9	25·7	28·6	31·5	34·3	37·2	40	42·9	45·8	48·6	51·5	54·3
N. glauca	24	—	—	15	22	41	14	6	2	—	—	—	—
N. Langsdorffii	18	2	9	51	22	16	—	—	—	—	—	—	—
N. Sanderae	18	3	15	44	26	5	6	—	—	—	—	—	—
(*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii*	30	1	2	2	8	5	12	9	6	2	3	—	—
(*N. glauca* × *N. Langsdorffii*) × *N. Sanderae*	30	—	—	—	1	6	5	34	41	13	—	—	—
Amphidiploid *N. glauca* × *N. Langsdorffii*	42	—	—	—	—	—	—	2	6	3	3	5	4

Forms	Somatic chromosomes	57·2	60·0	62·9	65·8	68·6	71·5	74·4	77·2	80·0	*M*	*n*
N. glauca	24	—	—	—	—	—	—	—	—	—	32·86	100
N. Langsdorffii	18	—	—	—	—	—	—	—	—	—	27·77	100
N. Sanderae	18	—	—	—	—	—	—	—	—	—	28·44	100
(*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii*	30	—	—	—	—	—	—	—	—	—	37·12	50
(*N. glauca* × *N. Langsdorffii*) × *N. Sanderae*	30	—	—	—	—	—	—	—	—	—	41·38	100
Amphidiploid *N. glauca* × *N. Langsdorffii*	42	18	3	18	9	3	16	1	2	1	60·52	94

TABLE XII

Breadth of the stomata board cells in microns

Forms	Somatic chromosomes	Breadth in microns								*M*	*n*
		8·6	11·4	14·3	17·2	20	22·9	25·7	28·6	31·5		
N. glauca	24	9	48	38	5	—	—	—	—	—	12·56	100
N. Langsdorffii	18	—	23	73	4	—	—	—	—	—	13·76	100
N. Sanderae	18	14	74	12	—	—	—	—	—	—	11·38	100
(*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii*	30	1	6	24	15	3	1	—	—	—	15·20	50
(*N. glauca* × *N. Langsdorffii*) × *N. Sanderae*	30	—	—	—	13	50	24	13	—	—	20·90	100
Amphidiploid *N. glauca* × *N. Langsdorffii*	42	—	—	1	14	15	32	16	12	10	20·49	100

TABLE XIII

Diameter of the P.M.C. during the tetrad stage (ocular micrometer divisions)

Forms	Somatic chromosomes	21	22	23	24	25	26	27	28	29	30	n	M	σ
Amphidiploid *N. glauca* × *N. Langsdorffii*	42	1	0	7	9	27	19	19	9	2	7	100	26·00	1·83
(*N. glauca* × *N. Langsdorffii*) × *N. Sanderae*	45	—	1	7	5	30	18	20	14	0	5	100	26·03	1·7

TABLE XIV

Diameter of the viable pollen grains in microns (measurements carried out in 1933)

Forms	Somatic chromosome numbers	Diameter in microns: 23·9	25·7	28·6	31·5	34·3	37·2	40	42·9	45·7	48·6	51·5	54·3	57·2	60·0	M	n
(1) *N. glauca*	24	—	—	20	66	24	—	—	—	—	—	—	—	—	—	31·6	110
(2) *N. Langsdorffii*	18	1	38	64	18	—	—	—	—	—	—	—	—	—	—	27·9	121
(3) *N. glauca* × *N. Langsdorffii*	21	—	—	4	20	42	46	58	32	5	3	1	—	—	—	37·9	211
(4) (*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii*	30	—	5	33	90	70	17	3	—	—	—	—	—	—	—	32·3	218
(5) Amphidiploid (F_2) *N. glauca* × *N. Langsdorffii*	42	—	—	—	3	19	92	85	26	—	—	—	—	—	—	37·7	225
(6) (*N. glauca* × *N. Langsdorffii*) × *N. Sanderae*	30	—	—	8	42	52	45	28	4	1	2	6	4	5	1	36·5	198
(7) (*N. glauca* × *N. Langsdorffii*) × *N. Sanderae*	45	—	—	4	8	39	87	66	42	27	23	16	18	9	12	44·5	331
(8) *N. Sanderae*	18	—	—	138	62	—	—	—	—	—	—	—	—	—	—	29·4	200
(9) (*N. glauca* × *N. Langsdorffii*) × *N. Langsdorffii* forming dyads	30	—	1	21	35	33	37	27	12	2	6	7	9	12	9	39·1	211

to a significant increase of the size of the cells (Tables XI–XV). Aneuploid chromosome alterations do not necessarily alter the size of the cells, though in some cases they do. The average size of the viable pollen grains in F_1 hybrids was almost equal to the size of the pollen grains of the amphidiploid because only those of the pollen grains were viable which originated from dyads having 21 (rarely 21 ± 1) i.e. the same chromosome number that the pollen grains of the amphidiploids have (Table XIV). F_1 hybrids formed a very small percentage of monads. It seems that the largest pollen grains (*ca.* 50 μ in diameter) have originated from monads. Pollen grains as large as 50 (± 2) μ in diameter were not found among the pollen of a large number of amphidiploid plants.

Triple hybrids mono-*glauca*—mono-*Langsdorffii*—mono-*Sanderae* ($2n = 30$) and the triple hybrid with 45 somatic chromosomes formed viable pollen, very variable in size. Both hybrids often formed dyads. Trigenomal

TABLE XV

Diameter of the pollen grains in microns (measurements carried out in 1938)

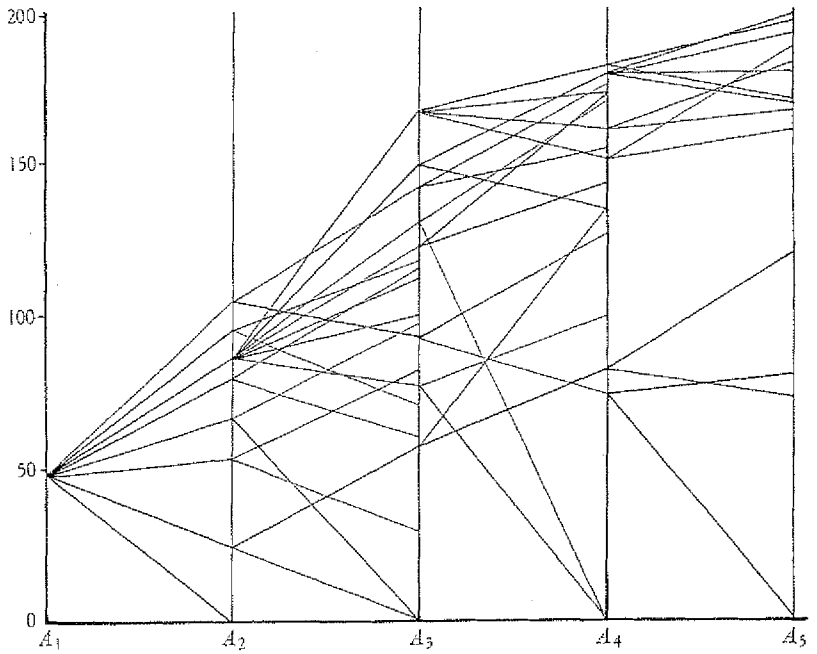
Forms	Somatic chromosome number	Diameter in microns 24·3–25·7	27–28·4	29·7–31	32·4–33·8	35·1–36·5	37·8–39·2	40·5–42	*n*	*M* in microns
N. glauca	24	—	20	48	26	—	—	—	94	30·3
N. Langsdorffii	18	10	27	31	2	—	—	—	70	28·3
Amphidiploid *N. glauca* × *N. Langsdorffii* F_6	42	—	—	—	24	40	62	33	159	37·5

hybrids mono-*glauca*—di-*Langsdorffii* ($2n = 30$), which did not form dyads, or formed them very rarely, had pollen size as that given in Table XIV (4), while the same trigenomal hybrids, that formed dyads especially when grown in abnormal conditions (6–8° C.), had a larger average diameter of pollen, because they formed also viable pollen with 60 chromosomes. Such an example is given in Table XIV (9). Pollen diameter of the amphidiploids of F_2 generation (Table XIV) was equal to that of the amphidiploids of F_6 generation (Table XV).

FERTILITY OF THE AMPHIDIPLOIDS

Fertility of the amphidiploids depends chiefly on the viability of the gametes formed by them. The original amphidiploid had a very reduced fertility, but it had a small percentage of viable pollen grains. With the increase of the number of generations the percentage of the viable pollen grains gradually increased (Tables IX and X). The increase of the number of seeds per capsule runs almost parallel with the increase of the percentage of viable pollen.

In studying the amount of seeds set per capsule by the original amphidiploid and its progeny throughout five generations I obtained data which are diagrammatically presented in Text-fig. 35. The average amount of seeds per capsule (separately for each plant studied) is given on the ordinate; the amphidiploid generations are given on the abscissa. The seeds were obtained by artificial selfing of the flowers. The original



Text-fig. 35. Diagram showing the degree of fertility of the first amphidiploid plant (A_1) and of its progeny (A_2, A_3, A_4 and A_5). Abscissa: the number of generations. Ordinate: average number of seeds per capsule of single plants. Note the gradual increase of fertility with the increase of generations.

amphidiploid set about 28 seeds per capsule when it was not artificially self-pollinated, and about 48 seeds per capsule when the flowers were self-pollinated. It formed about 51 % of viable pollen grains. The amount of seeds set by the plants in the F_2 generation depends chiefly on the genotypes of the plants. On selfing eight F_2 amphidiploid plants, one set about 106 seeds per capsule. It had 42 chromosomes and showed the highest fertility. Three other plants, also having 42 chromosomes, had respectively 92, 83 and 78 seeds per capsule. One plant, having 43 chromosomes, set about 65 seeds per capsule, another plant, having 44 chromosomes, set on the average 52 seeds per capsule. One plant,

having 44–45 chromosomes, set about 23 seeds per capsule. Another plant having the same chromosome number was self-sterile.

The progeny of these seven, self-fertile F_2 amphidiploid plants were grown on; several plants of each family were self-pollinated, and the seeds obtained were counted.

The largest amount of seeds per capsule was set by one plant, obtained from the F_2 plant with 83 seeds per capsule. One plant of the same family set less than 83 seeds per capsule. The most fertile plant in F_3 set about 167 seeds per capsule, while the most fertile plant of the family, grown from the most fertile one in F_2, set about 140 seeds per capsule. In F_4 generation the most fertile plant set about 182 seeds per capsule and in F_5 generation the fertility was increased to 199·5 seeds per capsule. On the other hand, highly fertile plants of F_2, F_3 and even of F_4 produced in the subsequent generations self-sterile plants. In other words *fertility increased for some amphidiploids gradually with the increase of the number of generations, but at the same time plants were segregated with low fertility* (even completely sterile ones). By selecting plants with highest fertility amphidiploids were produced during five generations that had an increased fertility from 48 seeds per capsule up to 200 seeds (exactly 199·5) per capsule. The pollen viability was also increased from 51 % to 98·5 %. I shall point out that the fertility "200 seeds per capsule and 98·5 % of viable pollen" had only single amphidiploid plants, while in the fifth generation, there were also amphidiploids with lower fertility and smaller percentage of viable pollen.

Three most important points of the long chain of causes and sequences that condition the degree of the fertility of the amphidiploids are: (1) the number of multivalents and univalents per cell formed by the amphidiploids; (2) the type of chromosome distribution during the first meiosis and the frequency of a 21 : 21 chromosome distribution; (3) the percentage of viable gametes.

Another factor that interferes with the fertility in the amphidiploids is the position of the stigma in respect to the position of the anthers. Amphidiploids that have styles 1–3 mm. longer than the longest anthers cannot often be self-pollinated without external aid (artificially by man, or by insects). Some of these plants set a very large amount of seed when artificially pollinated, but they have smaller chance of survival in nature than those of which the stigma is at one and the same level with the anthers. Some of the amphidiploids had much shorter styles, so that their stigmas were situated 1–3 mm. below the anthers. Such flowers occasionally set a reduced amount of seeds, when one compares the

differences between the numbers of seed sobtained from self-pollinations without external aid and artificial self-pollinations in this type of amphidiploids with those that had stigmas situated at the anther's level.

Alkaloid and citric acid contents in the amphidiploids of the fifth generation

The great morphological variability of the amphidiploids *N. glauca*—*N. Langsdorffii* described in this paper is associated with certain biochemical changes in the plant organism. It seemed reasonable that the content of some chemical compounds in the amphidiploids should also vary. Biochemical analysis carried out for us in the Biochemical Laboratory of the Institute of Genetics under the direction of Dr A.

TABLE XVI

Alkaloid content in F_5 amphidiploids and in the parental species

Plants	Alkaloid content %
N. glauca	1·041
N. Langsdorffii	1·253
Amphidiploids: plant No. 75014 (4)	**1·819**
No. 75014 (100)	1·350
No. 75014 (101)	1·495
No. 75014 h (104)	1·182
No. 75014 h (106)	0·781
No. 75014 h (107)	1·337
No. 75014 h (108)	1·432
No. 75014 h (112)	**0·706**
No. 75014 h (113)	0·926

Shmuck showed that the alkaloid and the citric acid (in form of various salts) contents were very different in different plants. Parental forms grown under the same environmental conditions gave 1·041 % (*N. glauca*) and 1·253 % (*N. Langsdorffii*) alkaloid content, while one of the amphidiploid plants had as high as 1·819 % (the highest) and another as low as 0·706 % (the lowest) alkaloid content. The other amphidiploids studied had lower than the highest and higher than the lowest alkaloid content (Table XVI).

The citric acid content varied much more than the alkaloid content. *N. glauca* had 3·760 %, *N. Langsdorffii*—4·603 % while the citric acid content of the amphidiploids varied between 1·524 % [plant 75014 (2)] and 6·515 % [plant 75014h (15)], i.e. somewhat more than four times (Table XVII). Plants with so high a citric acid content might be used for production of this compound.

TABLE XVII

Citric acid content in F_5 amphidiploids N. glauca—N. Langsdorffii *and in the parental species*

Plants	Citric acid content %
N. glauca	3·760
N. Langsdorffii	4·603
Amphidiploids:	
Plant No. 75014 (1)	2·489
No. 75014 (2)	**1·524**
No. 75014 (3)	2·142
No. 75014 (4)	**1·614**
No. 75014 (100)	2·070
No. 75014 h (104)	6·086
No. 75014 h (106)	**5·984**
No. 75014 h (107)	4·280
No. 75014 h (108)	3·673
No. 75014 h (112)	**5·098**
No. 75014 h (113)	2·222
No. 75014 h (113*a*)	3·978
No. 75014 h (114)	4·369
No. 75014 h (115)	**6·515**

Remark: Plant 75014 h (115) had *ca.* four times greater percentage of citric acid in form of salts than plant 75014 (2).

EVOLUTIONARY SIGNIFICANCE OF THE AMPHIDIPLOIDS AND IN PARTICULAR THE AMPHIDIPLOIDS *N. GLAUCA*—*N. LANGSDORFFII* (*N. VAVILOVII*)

The hypothesis advanced by Winge (1917), that species with polyploid chromosome numbers might have originated like *Primula kewensis* has been proved by many examples. Synthesis of *Galeopsis tetrahit*, (Müntzing, 1932*a*) from *G. pubescens* and *G. speciosa*, *Phleum pratense hexaploidum* (Gregor & Sansome, 1930) from *P. pratense* and *P. alpinum*, *Prunus domestica* (Rybin, 1936) from *P. divaricota* and *P. spinosa*, *Rubus maximus* (Rosanova, 1938) from *R. Idaeus* and *R. caesius*, *Nicotiana tabacum* (Kostoff, 1936*a*, 1938*d*) from *N. sylvestris* and *N. tomentosiformis*, etc., can be given here as good examples. On the other hand we have good evidence that autopolyploidy has also played a very important role in evolution. I may refer to the excellent paper by Müntzing (1936) upon this subject. Wulff (1937), on the other hand, discussed broadly the geographical distribution of the polyploid plants when considering the recent investigations by Hagerup (1932) and Tischler (1935) upon this subject. Cytogenetics of the autopolyploids (including haploids) and allopolyploids have been thoroughly discussed by Darlington (1932, 1937), Karpetchenko (1935*a*, 1935*b*) and Kostoff (1938*b*, 1938*j*, 1938*k*), therefore I shall consider here chiefly the allopolyploids and their deri-

vatives as a suitable material for giving rise to new species after undergoing the natural selection. The amphidiploid *N. glauca—N. Langsdorffii* will serve as an example.

Before evaluating the inconstant amphidiploids from an evolutionary point of view I may first call attention to the so-called "constant" allopolyploids which have originated from F_1 hybrids with asyndetic meiosis. I shall consider here the amphidiploid, obtained from the F_1 hybrid *Nicotiana multivalvis* ($n=24$) × *N. suaveolens* ($n=16$) having asyndesic meiosis (Kostoff, 1937*c*). The geographical distribution of the maternal plant is North America, while that of the paternal one is Australia. The amphidiploid of these two widely separated species is practically constant. Amphidiploid plants have normal meiosis, *ca.* 98–99 % of viable pollen, set a larger amount of seeds per capsule than *N. suaveolens* and form many more flowers than *N. multivalvis*. It is

TABLE XVIII

Average number of seeds per capsule and capsules per plant for a vegetation period. Plants grown in the greenhouse at equal conditions

Plants	Seeds per capsule	Capsules per plant	Total seeds per plant
N. multivalvis ($n=24$)	456	2·8	1276·8
N. suaveolens ($n=16$)	87	11·7	1017·9
Amphidiploid:			
N. multivalvis—N. suaveolens ($n=40$)	127	10·9	1384·3

almost immune to most of the virus and other diseases that affect *Nicotiana* species and varieties in our conditions like *N. suaveolens*, while the maternal species is highly susceptible. All these positive characters would probably secure a survival of the amphidiploid in natural conditions (Table XVIII).

But since this amphidiploid is highly constant it might give rise to a *monomorphic species* when undergoing natural selection.

There is no doubt that the amphidiploids, originating from F_1 hybrids with partial or complete allosyndesis, might give rise to very variable populations, i.e. they might supply more suitable material for natural selection.

The numerous high fertile amphidiploid forms *N. glauca—N. Langsdorffii*, originating from amphidiploid and highly fertile aneuploid types, do not yet represent a new species, but this abundance of forms might with time give rise to a new *polymorphic species*, since a large number of these forms can survive in the struggle for existence.

Production of fully fertile forms from the partially fertile original

amphidiploid is not simply due to chromosome rearrangements, since numerous gene mutations have undoubtedly occurred. Those of them that condition harmonious development and increased fertility have been selected, while those leading to disharmonious development and low fertility have been eliminated (lethality).

As to the frequency of mutations I may recall Baur's observations (1924) who estimated the rate of the small mutations at about 10 %. This kind of mutation is, undoubtedly fundamental in evolution. They are recombined by hybridization. I may also recall here our data (1935 *b*) for *Nicotiana* species hybrids, as well as those of Belgovsky (1934) for *Drosophila*, which showed that the mutation rate in species hybrids is increased. Some of the data presented here, and those recorded in earlier publications, showed also that chromosome alterations occur more frequently in species hybrids when compared with the mutation rates of the parental species (Kostoff, 1938 *b*, 1938 *c*). On the other hand our recent investigations (Kostoff, 1938 *k*) showed that amphidiploids and allopolyploids represent quite new systems in many respects. The pure mechanical process "chromosome doubling" leads to a series of changes in the trends of the formative reactions in the polyploids. I may recall here the characters, as increase of the breadth of the leaves, thickness of the leaves, size of the nuclei, cytoplasm, cell size, etc. (Kostoff, 1938 *k*) and the autonomy (i.e. no significant alteration in the size) of the chloroplast when a euploid chromosome alteration (polyploidy or haploidy) occurs (cf. Kostoff & Orlov, 1938).

It is logical to expect that the mutation rate induced by the external factors in such new polyploid systems cannot be identical with that of the original forms.

The numerous chromosome alterations and gene mutations that have occurred in the original amphidiploid and in its euploid and aneuploid derivatives have been selected or eliminated—depending on their degree of fitting—as pollen-grains (germination), as pollen tubes (rate of growth), as egg cells, as zygotes, as young embryos, as small plants, as adult plants (degree of fertility), etc.

Chromosome rearrangement, as could be stated with certainty for the satellite chromosome, might also have occurred in some of the other chromosomes. This kind of rearrangements leads to the formation of euploid forms with new karyotypes, and facilitates the formation of aneuploid forms with new, relatively constant or oscillating karyotypes.

Forms with new karyotypes resulting from interspecific hybridization in *Crepis* were reported by Babcock & Emsweller (1936). Forms with

changed constant aneuploid karyotypes were obtained by Blakeslee and his co-workers (1936, 1937) in *Datura*, from material treated with X-rays and radium. Large numbers of aneuploid forms of specific range were obtained by J. Clausen (1932) in *Viola*, by means of hybridization. Good species, as for example *Poa alpina* and *P. pratensis*, have oscillating chromosomes. In the former species they oscillated between 22 to 38. Müntzing (1932*a*) found thirteen different numbers for this species, eleven being aneuploid. *P. pratensis* behaves in a similar way. Müntzing found for this species seven biotypes with aneuploid and one with euploid chromosome number ranging from 64 to 85. Another species with oscillating chromosome number is *Viola canina* described by Clausen (1931). This species has $2n = 40 + a$ varying number of extra-chromosomes or fragments.

Many of the highly fertile aneuploid forms of *N. glauca—N. Langsdorffii* hybrids with oscillating chromosome numbers might survive along with the euploid ones.

Findings of rearrangements in one or more than one chromosome in the progeny of interspecific hybrids supply convincing evidence that new forms with new karyotypes might originate from structural hybrids (Text-figs. 33 and 34). The studies of chromosome morphology in the somatic cell for detecting chromosome alterations is a relatively rough method since numerous small rearrangements cannot be detected in this way.

In studying the type of chromosome conjugations during the meiosis in wheat hybrids between extracted derivatives from structural interspecific hybrids and the original parental forms, I found a series of new rearrangements that were not possible to detect from the morphology of the chromosomes (Kostoff, 1937, and unpublished). This kind of study offers a solid background for estimating the role of structural interspecific hybrids in evolution.

Euploid as well as aneuploid forms of *N. glauca—N. Langsdorffii* hybrids are physiogenetically isolated from the parental species as well as from the other *Nicotiana* species and amphidiploids (Table XIX). Either they do not cross, or hybrids obtained from their crosses are sterile. The crosses with *N. glauca* generally failed, because the pollen tubes usually do not reach the ovary whichever way the cross is made. The crosses amphidiploid × *N. Langsdorffii* failed, because the hybrid embryos grow very slowly and the seeds obtained do not germinate. The cross *N. Langsdorffii* × amphidiploid failed because the pollen tubes of the amphidiploid do not reach the ovary of *N. Langsdorffii*.

DISCUSSION AND CONCLUSION

The data obtained from the cytogenic investigations carried out with *N. glauca—N. Langsdorffii* hybrids during the last ten years together with some of those contributed recently by other authors in the same line may serve as a starting point for a series of general statements. Since the limit of this paper does not allow me to consider here all questions that arise in connexion with the data presented in this paper, I shall discuss only those of them that are not and will not be broadly discussed elsewhere.

(1) *Crossability.* Our data suggested that environmental conditions, genotypes (also numerical and structural karyotypes), and the age of the plant are responsible, in various degrees, for the species crossability. Temperature is one of the factors that influences both pollen-tube growth and embryo development. The chromosome number is also a factor on which depends the rate of the pollen-tube growth. Thicker pollen-tubes (having larger chromosome number) grow more slowly through styles with smaller chromosome numbers, than thinner pollen tubes through styles with larger chromosome numbers (cf. Kostoff, 1934*c*, Kostoff & Prokofieva, 1935).

(2) *Irregular mitosis in species hybrids.* The hybrids *N. glauca—N. Langsdorffii*, as well as some other species hybrids, showed higher frequency of abnormal mitosis than the parental species. It seems to me that two different kinds of process are responsible for the occurrence of this phenomenon.

A. The velocities of various reactions that represent single links of a series of reactions responsible for a certain biological process in one species differ from that in another. It seems that in our particular case the velocities of the reactions, responsible for the procedure of the mitotic processes in *N. glauca* do not quite coincide with the velocities of the reactions that condition the respective processes in *N. Langsdorffii*. When these processes conditioned by the genetic contributions of the parental species are not quite harmoniously summed in the F_1 hybrids, but a certain discordance occurs resulting from the interference between the mitotic processes regulated by the parental genetic contributions, abnormal mitosis may appear. This conception is diagrammatically represented in Text-fig. 36 which shows the procedure of a chain of reactions in one species (A_1, B_1, C_1, etc.) and in another (A_2, B_2, C_2, etc.). Since the velocities of the reaction development in the one species (straight line I) differ from those of the other (broken line I), their trends might diverge

TABLE XIX

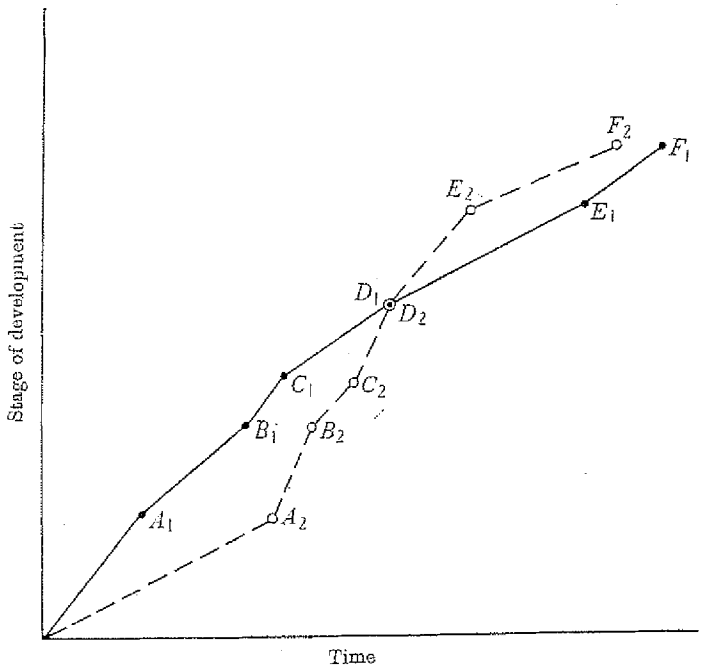
Crossability of the euploid and aneuploid forms of the amphidiploid N. glauca—N. Langsdorffii *crossings carried out* 1937 *in the greenhouse (July, August)*

Maternal plant	2n	Paternal plant	2n	Flowers pollinated	Capsules obtained	Hybrids raised	Average no. of ovules participating in the cross	Remarks
Amphidiploid *N. glauca—N. Langsdorffii*	42	*N. rupa* (amphidiploid *N. rustica—N. paniculata*)	72	62	0	—	17120	—
N. rupa	72	Amphidiploid *N. glauca—N. Langsdorffii*	42	43	40	7	6080	Hybrids sterile when free flowering
Amphidiploid *N. glauca—N. Langsdorffii*	42	Amphidiploid *N. rustica—N. tabacum*	96	75	2	3	19500	Hybrids self-sterile
Amphidiploid *N. glauca—N. Langsdorffii*	42	Amphidiploid *N. multivalvis—N. suaveolens*	80	95	3	2	24700	,,
Amphidiploid *N. rustica—N. tabacum*	96	Amphidiploid *N. glauca—N. Langsdorffii*	42	49	27	12	14553	,,
Hyperamphidiploid *N. glauca—N. Langsdorffii*	44	*N. rupa*	72	36	0	—	9360	—
Amphidiploid *N. glauca—N. Langsdorffii*	42	*N. glauca*	24	10	—	—	2600	—
Amphidiploid *N. glauca—N. Langsdorffii*	42	*N. Langsdorffii*	18	10	8	0	2600	—
Hyperamphidiploid *N. glauca—N. Langsdorffii*	44	*N. glauca*	24	10	0	—	2600	—
N. glauca	24	Amphidiploid *N. glauca—N. Langsdorffii*	42	20	0	—	16100	—
N. Langsdorffii	18	Amphidiploid *N. glauca—N. Langsdorffii*	42	20	0	—	3960	—
N. glauca	24	Hyperamphidiploid *N. glauca—N. Langsdorffii*	44	20	0	—	16100	—
N. Langsdorffii	18	Hyperamphidiploid *N. glauca—N. Langsdorffii*	44	20	0	—	3960	—
Amphidiploid *N. glauca—N. Langsdorffii*	42	*N. Sanderae*	18	20	12	0	5200	—
Hyperamphidiploid *N. glauca—N. Langsdorffii*	44	*N. Sanderae*	18	5	4	0	1300	—

(*A*, *E*), converge (*B*, *D*, *F*) or run almost parallel (*C*). Some ends and starts of reactions might coincide in time and stage of development (*D*).

The beginning and the end of certain reactions do not take place at exactly the same stage of development in different species. This introduces new disharmonies.

The interpretation advanced for explaining the causes of irregular mitotic processes serves also to explain the causes for the irregularities in



Text-fig. 36. Diagram showing theoretically the duration (time) of development of biochemical reactions in two different (1 and 2) species in respect to various developmental stages. Some of extremely divergent or convergent trends might condition abnormal (discordant) processes in the F_1 hybrids.

the meiosis of amphidiploids originating from F_1 hybrids with asyndetic meiosis, and for the gradual increase of fertility with the increase of *N. glauca—N. Langsdorffii* amphidiploid generations to be referred to later.

B. Another type of irregularities in meiosis seems to result from exchange of parts between the chromatids of the somatic chromosomes (cf. Stern, 1936; Kostoff, 1938*b*). This probably occurs between homologous segments as well as between non-homologous one, most probably in the heterochromatic regions of the latter (Kostoff, 1938*b*). External

factors (X-rays, temperature) often induce interchanges between non-homologous chromosomes. It seems that interspecific hybridization sometimes favours chromosome exchanges. Somatic anaphase with chromatin bridges observed in the F_1 hybrid *N. glauca—N. Langsdorffii* as well as those observed in the tapetum cells of F_1 hybrids *N. bonariensis* × *N. Sanderae* (Kostoff, 1938*b*) can be explained by postulating exchange of parts between the chromatids of two somatic chromosomes. We might suppose that the exchange in *N. glauca—N. Langsdorffii* hybrid which led to formation of a chromatin bridge has occurred between homologous segments if we found the same bridges during meiosis, certifying an inversion. But chromatin bridges were not found during meiosis, consequently chromatin bridges during the mitosis must have resulted from an exchange between non-homologous segments, most probably between heterochromatic regions.

(3) *Non-parasitic tumours formed by species hybrids.* Abnormal mitosis occurred quite frequently in the tumorous malformations developed by the hybrids *N. glauca—N. Langsdorffii*. Polyploidy or aneuploidy does not seem to be the cause for the tumour formation since I have most frequently found in the tumour tissues the normal somatic chromosome number. On the other hand I have raised tetraploid plants from *N. glauca* and numerous aneuploid from *N. Langsdorffii*. None of them developed tumours. Consequently the increase of abnormal mitosis in tumours which leads to formation of polyploid and aneuploid cells in tumour tissues is rather a sequence from the same cause or causes that condition tumour formation. The hypothesis for tumour formation advanced by Whitaker (1934) and adopted by Levine (1936) that tumours in *N. glauca—N. Langsdorffii* hybrids as well as in all *Nicotiana* hybrids is necessarily connected with chromosome number "9", does not hold, because I have raised species hybrids from the cross combinations: *N. rustica* ($n=24$) × *N. Cavanillesii* ($n=12$), *N. glauca* ($n=12$) × *N. longiflora* ($n=10$), etc., and all of them formed tumours.

If tumour formation results from certain chromosome alterations, it should be then most probably conditioned by somatic chromosome exchanges (cf. Kostoff, 1938*b*). I shall mention here that Jones (1936, 1937) is also inclined to interpret certain kinds of atypical growth by somatic chromosome rearrangements.

At present the most probable interpretation for non-parasitic tumour formation in F_1 hybrids, euploid and aneuploid hybrids of *N. glauca—N. Langsdorffii*, as well as in other *Nicotiana* species hybrids, seems to be the somatic interchange hypothesis (Kostoff, 1938*b*). The interchanges

that condition atypical growth seem to occur most frequently when the plant is old.

The time of appearance of non-parasitic tumours in *N. glauca—N. Langsdorffii* and in other *Nicotiana* hybrids as well as the histology and cytology of these tumours resembles very much the cancerous atypical growth in man and in animals; hence the physiology and the biochemistry of the plant tumours have recently formed the subject of a series of investigations. The production of amphidiploid *N. glauca—N. Langsdorffii* hybrids allows us to propagate the hybrids forming tumours in an unlimited number, and facilitates the supply of physiological and biochemical investigations with large amount of material. These amphidiploids represent the most convenient object for studying the physiology and biochemistry of atypical growth. (One single large plant might form about 25–30 g. of tumours in favourable conditions).

E. Stein (1930–7) has published a series of papers in which she claimed that she has succeeded in obtaining a strain in *Antirrhinum* by radium treatment which forms "phytocarcinomes". Actually her strain forms occasionally single cells, or groups of cells, with increased chromosome number. Looking over the photographs of her plants forming "phytocarcinomes" one cannot find real cancerous outgrowths. They do not form visible tumour proliferations like those given in our figures. What she has really produced is a strain that not infrequently forms one or a few cells with increased chromosome numbers at various places, which have obviously the tendency to die off somewhat earlier than the diploid cells, without killing the plants. The cells of which she gives microphotographs resemble somewhat the polyploid cells in various plants originating under the influence of acenaphtene and colchicine (Ludford, 1936; Kostoff, 1938*e*, *f* and unpublished; Levan, 1938; Walker, 1938, etc.) as well as the tapetum cells. She also describes cancerous degeneration in tapetum cells. If one assumes the abnormal cells she has found for "phytocarcinomes", one must then logically assume that each individual of the higher plants forms "carcinomes", since the tapetum cells of each plant are like those she calls "Krebsentartung" tissue. Tapetum cells expand enormously, their nuclei divide, the chromosomes often do not separate, thus forming large, easily degenerating polyploid cells of various degrees (4*n*, 8*n*, and many more). When the chromosomes get somewhat separated but cytokinesis fails, they become multinucleate (Kostoff, 1930*a*, 1938*b*, also unpublished). I doubt, however, whether botanists, histologists, and especially cancerologists would call phytocarcinomes a "Krebsentartung" of the tapetum cells that takes place as

a rule in each plant. (I have found degeneration of tapetum cells in each plant studied out of 45 *Nicotiana* species and 126 species hybrids.)

If one calls the polyploid cells or group of cells that are occasionally formed by E. Stein's *Antirrhinum* strain for carcinomes, one must then also admit that each mosquito suffers from cancer, since the cells of the alimentary tract of mosquitos are polyploid in various degrees. These arguments as well as the absence of real tumour outgrowths on the plants of her "carcinome" forming strain show that Stein's strain cannot be classified with the plants forming hereditary non-parasitic "phyto-carcinomes".

(4) *Meiotic irregularities.* Meiotic irregularities in species hybrids are chiefly due to structural and numerical chromosome differences in the parental species. But in studying the meiosis of the amphidiploids *N. multivalvis* × *N. suaveolens* and *Secale montanum* × *Triticum durum* obtained from F_1 hybrids with asyndetic chromosome behaviour, I occasionally found in them univalent chromosomes during the first meiotic metaphase although "numerical" and "structural" differences in them were eliminated by chromosome doubling. Lewitzky & Benetzkaya (1929) also found univalents in the amphidiploid *Triticum vulgare—Secale cereale* (F_1 hybrids of this cross combination have usually asyndesis). The behaviour of these three amphidiploids suggests that for the irregularities in meiosis (in this particular case, for the appearance of univalents, i.e. reduced pairing and sometimes failure of chiasma formation between certain pairs) other factors should be responsible than those mentioned above. During the last decade numerous genetic and a series of cytogenetic phenomena were explained by postulating unknown functions of the cytoplasm. The easiest way to interpret the irregular meiosis in amphidiploids originating from F_1 hybrids with asyndesis would be to postulate incongruence between the cytoplasm of the maternal species and the chromosomes of the paternal one. Such speculations, however, have no scientific basis since we do not know yet the degree of autonomy of the cytoplasm or the kind and quantity of cytoplasm that is brought into the embryo-sac by the pollen-tube of the paternal species. Therefore I am inclined to assume another, more probable interpretation, that is connected with the developmental processes in the parental species. In the above mentioned amphidiploids meiosis proceeds at somewhat different stages of development, namely meiosis in *Secale* proceeds about 3–7 days (roughly estimated) later than in *Triticum*. In other words, hereditary units of the genom and plasm of *Secale* condition meiosis in this genus somewhat later than those of *Triticum* in *Triticum*. One

cannot yet decide whether *Triticum* genotype more rapidly produces substances necessary for the meiotic processes, or whether both genera produce at the same stage of development the same amount of substances that regulate meiosis; but in *Secale* cells, meiosis can proceed, when a greater quantity of these substances is accumulated. Hence, when pairing and crossing-over for *Triticum* chromosomes proceed, so to say, in normal milieu, the same processes are somewhat premature for *Secale* chromosomes, so that pairing and crossing-over (chiasma formation) for them is reduced, the extreme—being a complete failure of pairing of certain pairs, no chiasma formation, and further—univalency. Such development of biochemical reactions conditioning meiotic processes seems very probable. A part of the univalents that appeared in the amphidiploid *N. glauca—N. Langsdorffii* can be also interpreted by lack of coincidence of certain processes regulated by *N. glauca* and *N. Langsdorffii* genotypes. This can be attributed only to a part of the univalents, because it was very probable that some of them resulted from interference of chiasmata when trivalents were formed.

How then can the fact be explained that, within six generations, amphidiploids with almost normal meiosis were obtained? It seems to me, that two factors are chiefly responsible for this phenomenon, namely: (1) a greater structural differentiation of the chromosomes, and (2) a greater genic differentiation and accumulation of the mutations that secure a greater harmony in the discordant processes (in time as well as qualitatively and quantitatively) of the amphidiploid.

(5) *Monomorphic and polymorphic species originating from amphidiploids.* High constancy of the amphidiploids originating from hybrids with asyndetic meiosis and high fertility suggest that such allopolyploids should give rise to monomorphic species, while the amphidiploids of the type *N. glauca—N. Langsdorffii* should give rise to polymorphic species. The latter should also give rise to aneuploid forms and aneuploid species as well as to species with oscillating chromosome numbers.

It is also possible that in certain cases amphidiploids might arise from different varieties of two different species AA and BB, namely: $A_1\,A_1\,B_1\,B_1$, $A_1\,A_1\,B_2\,B_2$, $A_2\,A_2\,B_1\,B_1$, $A_2\,A_2\,B_2\,B_2$, $A_1\,A_1\,B_5\,B_5$, etc., which might further intercross and increase the polymorphism of the species (cf. Rosanova, 1938), but it seems more probable that the polymorphism of the allopolyploid species is rather due to the factors that conditioned polymorphism in the progeny of *N. glauca—N. Langsdorffii* amphidiploid described in this paper. It seems that the constancy of the amphidiploids is very questionable. Since I discussed this question in a previous paper

(Kostoff, 1935) it will be considered here very briefly. The process of meiosis in the majority of the amphidiploids recorded by various authors suggests that they should not be constant, and most of them actually produced inconstant progeny (Buxton & Newton, 1928; Poole, 1932; Müntzing, 1934, 1935; Kostoff, 1935*a*, 1936*c*, 1937*c*, etc.). *Raphanus-Brassica* intergeneric amphidiploid (Karpetchenko, 1928) was considered as the best example of constant amphidiploids. The data reported by Richharia (1937) and those by Howard (1938) show that their *Raphano-Brassica* amphidiploids were not constant. The most constant amphidiploid that I know is that produced between North American tobacco *N. multivalvis* ($n=24$) and Australian *N. suaveolens* ($n=16$) (Kostoff, 1937*c*). Recently I produced another amphidiploid between Australian and American species, namely, *N. suaveolens* ($n=16$) × *N. alata* ($n=9$) by colchicine treatment, the meiosis of which, and of the F_1 hybrids, indicate that this amphidiploid should be highly constant, but perhaps less so than the *N. multivalvis*—*N. suaveolens* amphidiploid. This problem I have also considered in my earlier publications (Kostoff, 1937*c*, 1938*c*, *j*). It should also be noticed here that I have briefly discussed only a small part of the problem of species monomorphism and polymorphism, namely that connected with the degree of constancy of allopolyploids. The whole problem will be later considered elsewhere.

Summary

1. Parental species participating in the crosses were: *Nicotiana glauca* ($2n=24$), *N. Langsdorffii* ($2n=18$) and *N. Sanderae* ($2n=18$). The chromosome morphology of the first two species is given. The chromosome numbers that occur in the genus *Nicotiana* are also mentioned. The process of meiosis in *N. glauca* and *N. Langsdorffii* was studied. Abnormal meiosis, induced by acenaphthene in these two species is also described.

2. Hybrids from the cross *N. glauca* × *N. Langsdorffii* can be much more easily produced than from the reciprocal one. Environmental conditions and obviously the age of the plant influence the crossability. Crosses carried out in early spring and autumn between older plants are more successful than those carried out in summer between young plants. Pollen-tubes of *N. Langsdorffii* reach the ovary of *N. glauca* much more easily than *N. glauca* pollen-tubes the *N. Langsdorffii* ovary.

(3) Some crosses give quite normal F_1 hybrids while others give normal ones and dwarfs in a ratio 3:1. Most of the characters show an intermediate appearance in F_1 hybrids. Small trichomes of *N. Langsdorffii* appeared in F_1 with the same intensity. The bluish-violet colour of

N. Langsdorffii pollen is diluted in F_1. Each F_1 hybrid forms non-parasitic tumours. The latter usually appear when the plant is old, i.e. after the first florescence period of the main stem. Some F_1 plants formed tumours at an earlier stage, but such cases were less frequent. Tumours were formed by *N. glauca* × *N. Langsdorffii* as well as by the reciprocal hybrids. By grafting hybrids on the parental species and vice versa, tumours were formed only by the hybrid tissues.

4. F_1 hybrids have usually 21 somatic chromosomes. About 2 % of the mitotic figures were abnormal. The absence, or rather rare occurrence, of dividing cells with larger or smaller chromosome number than 21, indicates that the aberrant cells formed during the abnormal procedure of mitosis have a lower division rate, consequently they cannot usually compete with those having the normal chromosome number (21). Mitotic plates with 22, 23, 28, 42, etc., chromosomes occur very rarely and do not correspond to the abnormal mitotic anaphases found (2 %), although logically the cells with abnormal chromosome number should gradually increase with a division rate equal to that of normal ones.

5. Tumours formed by F_1 hybrids have usually 21 chromosomes. Regions with 42 chromosomes were also found. They occur most frequently near the necrotic regions. Cells with other chromosome numbers were rarely found. Tumour cells contained nutritive products, divided rapidly and usually do not become differentiated. They have small but numerous vacuoles, and occasionally many more nucleoli than the normal ones. The cells expand very rapidly and are easily affected by necrotic processes. The nuclei in these regions were deformed. Polyploidy and aneuploidy are not the cause of tumour formation, but are probably due to the cause or causes conditioning tumours.

6. *N. glauca* chromosomes have small segments homologous with portions of *N. Langsdorffii* chromosomes. Meiotic processes in F_1 hybrids *N. glauca* × *N. Langsdorffii* are regulated by the parental genotypes and influenced by external factors. From one up to nine bivalents per cell were found. F_1 dwarf hybrids had on the average 3–4 bivalents per cell; their sister plants that developed normally had 6–7 bivalents per cell; some other F_1 hybrids had even a larger number of bivalents per cell. Bivalents resulted from allosyndetic pairing and were usually held by one chiasma. Separation occurs by gradual terminalization of chiasmata. Asymmetric (heteromorphic) bivalents consisting of a large and a small chromosome were often formed. Trivalents were also formed.

7. Exchanges of parts in the bivalent and trivalent groups during the meiosis of F_1 hybrids following allosyndesis and, in exceptional cases,

following autosyndesis between homologous segments of the partially homologous chromosomes, and probably between heterochromatic regions of non-homologous or partially homologous chromosomes, lead to formation of chromosomes with new genetic content.

8. About 4 % of the P.M.C. have at least one divided univalent in the F_1 hybrid.

9. The percentage of monad and dyad formation in F_1 hybrids depends on the genotype and on the environmental conditions. High and low temperatures increase the percentage of dyads. Hybrids with 17, 37 %, etc., of dyads were found. About 2 % of monads were counted in one plant.

10. F_1 hybrids form about 8 % viable pollen grains. The average diameter of the latter is as large as that of the amphidiploids.

11. Dyads formed have different genetic constitutions. Triploids produced in crossing $F_1 \times N.$ *Langsdorffii* ($2n = 30$) differed morphologically.

12. F_1 hybrids are self-sterile. A few seeds were produced when crossed back to *N. Langsdorffii* and to *N. Sanderae*.

13. The majority of the plants obtained on crossing F_1 with *N. Langsdorffii* had 30 somatic chromosomes, i.e. two *N. Langsdorffii* and one *N. glauca* genom (if one neglects the exchange of parts during the meiosis in F_1 hybrids). Some chromosome aberrants were also obtained.

14. A chromosome aberrant with 20 somatic chromosomes showed much abnormal mitosis and formed branches with 26 and with doubled chromosome numbers (40).

15. One amphidiploid *N. glauca*—*N. Langsdorffii* was obtained in the back-cross. It probably originated parthenogenetically from a "monad".

16. Mono-*glauca*—di-*Langsdorffii* plants all having exactly 30 chromosomes differed morphologically and had unequal fertility. They had not equal meiosis. One plant had 9–12 univalents, other four plants formed 10–14 univalents. They all formed trivalents and a very variable percentage of viable pollen (8–50).

17. On crossing mono-*glauca*—di-*Langsdorffii* hybrids ($2n = 30$), that formed dyads, with *N. glauca* ($2n = 24$) chromosome aberrants, two amphidiploids and one hybrid with 72 somatic chromosomes (tri-*glauca*—tetra-*Langsdorffii*) were obtained. The latter resulted from fusion of an egg cell with 60 chromosomes (failure of both meiotic divisions) and a normal *N. glauca* sperm.

18. No seeds were obtained on crossing parental species with pollen of the F_1 hybrids. Only one capsule was produced on crossing numerous

flowers of *N. glauca* with pollen of mono-*glauca*—di-*Langsdorffii* hybrids.

19. On crossing F_1 hybrids with pollen of *N. Sanderae* ($n=9$) mono-*glauca*—mono-*Langsdorffii*—mono-*Sanderae* hybrids, with 30 somatic chromosomes, chromosomal aberrants and one with *ca.* 51 somatic chromosomes were produced. The latter represents di-*glauca*—di-*Langsdorffii*—mono-*Sanderae* hybrid and originated from a "monad"—egg of F_1 hybrid and a normal sperm of *N. Sanderae*. Meiosis and fertility of these triple hybrids is described.

20. Meiosis of the original parthenogenetically obtained amphidiploid was studied. It formed bivalents, trivalents, quadrivalents and univalents; the last three led to abnormal meiosis and formation of unequal gametes numerically and structurally.

21. The original amphidiploid formed 51 % of viable pollen; plants of F_2 generation formed 59 %; plants of F_4 generation 94 %, and single plants of F_6 generation 99·5 %.

22. The original amphidiploid set 28 seeds per capsule when it was not artificially self-pollinated, and 48 seeds per capsule, when it was artificially self-pollinated. Fertility increased gradually in subsequent generations and in some F_5 plants reached about 200 seeds per capsule. *N. glauca* sets on the average about 805 seeds and *N. Langsdorffii* about 198 when grown under the same conditions in the greenhouse. The original amphidiploid plant formed about 260 ovules per capsule.

23. The amphidiploid *N. glauca—N. Langsdorffii* ($2n=42$) is not constant. It "segregates" in subsequent generations (F_2—F_6) giving rise to plants unequal cytogenetically, morphologically, physiologically and biochemically. The plants differed from each other in respect of leaf size and shape, flower size and shape, type of growth, vegetation period, pollen colour, and chromosome numbers. Plants with 21, 23, 41, 42, 43, 44, 45, 46, 47, 48, 49, *ca.* 50, 51 and 52 chromosomes arose in subsequent generations.

24. The original amphidiploid, as well as those of the subsequent generations ($2n=42$), had broader leaves and in most cases larger flowers, than the F_1 hybrids.

25. Amphidiploids and some of their aneuploid derivatives tended in subsequent generations to reproduce plants with the same chromosome numbers. A few plants did not obey this rule.

26. The number of multivalents and univalents in the amphidiploids decreases with the increase of the number of generations.

27. The percentages of second metaphases with 21 chromosomes and viable pollen grains increase with the increase of the number of genera-

tions (F_2—F_6) amphidiploids ($2n=42$). Some amphidiploids with 99·5 % viable pollen grains were raised in F_6.

28. Fertility of the amphidiploids increases with the increase in the number of generations.

29. Heterochromatic chromosome pairs and heterochromatic regions were revealed in the meiotic chromosomes during the first metaphase in an amphidiploid. Heterochromatic regions are chiefly located near the centromeres. Some pairs had small heterochromatic regions at the distal ends.

30. A haploid with 21 somatic chromosomes and a hyperhaploid with 23 somatic chromosomes originated parthenogenetically. The meiosis of the former resembled that of the F_1 hybrids, but it differed somewhat morphologically from them. Heteromorphic pairs (a larger and shorter chromosome) were found in the haploid, like those in the F_1 hybrids and like those that were occasionally found in the amphidiploids.

31. In an F_3 plant the long arm of the satellite chromosome of *N. glauca* was significantly reduced, while in an F_4 plant the short arm was significantly elongated, so that the chromosome had almost median constriction. A diagram is given to explain the origin of these chromosome changes.

32. Euploid chromosome alterations lead ultimately to changes in the nuclei and cell sizes. Each additional genom led to a significant increase in size. Aneuploid chromosome alteration might also condition changes in the nuclei and cell size, but such changes were not always significant.

33. Fertility of the amphidiploids and of their derivative forms depended chiefly on: (*a*) the number of multivalents and univalents formed during the meiosis, (*b*) on the percentage of the viable gametes formed by the plants. Euploid forms showed somewhat higher fertility. The fertility of the euploid as well as of the aneuploid forms gradually increased with the increase in the number of generations.

34. The alkaloid and citric acid contents of the F_5 generation of the amphidiploids, like the morphological characters, vary very greatly. One plant had about four times more citric acid (6·515 %) in form of various salts, than another one (1·52 %).

35. Definite changes in the satellite chromosomes were found in some derivatives, showing that fundamental rearrangements in the chromosomes occur in structural hybrids, all of them being of great evolutionary significance since they condition the isolation of the new forms in nature.

36. Amphidiploids originating from F_1 hybrids with asyndetic meiosis are highly constant. They might give rise to a highly monomorphic species

if they survive in the struggle for existence. Amphidiploids originating from F_1 hybrids with complete or partial allosyndesis are not constant, and might give rise to a highly polymorphic species when a series of segregated forms survive in the struggle for existence.

37. Amphidiploids and their derivatives represent new organisms in which the mutation rate may be different from that in the parental species. Considering the data that show an increase of the chromosomal alterations and gene mutations in species hybrids and the frequency of the so-called small mutations, it was postulated that such mutations and chromosome rearrangements are probably responsible for the gradual increase in the fertility of the amphidiploids.

38. Inconstant amphidiploids may give rise to a series of adaptable forms; hence in certain cases they may afford more suitable material for natural selection than the highly constant amphidiploids.

39. Amphidiploids and their derivatives are physiogenetically isolated. They cross either with difficulty or not at all with other species and species hybrids or with the parental species.

40. Causes for (*a*) the irregularities in meiosis and mitosis, (*b*) the formation of hereditary non-parasitic tumours, and (*c*) the origin of monomorphic and polymorphic species from amphidiploids are suggested and critically estimated.

41. Increased frequency of chromosome alterations and gene mutations in species hybrids, tumour formation in *N. glauca—Langsdorffii* hybrids and the increased crossability when they were old indicate a series of fundamental changes (physiological, cytogenetical, etc.) take place in hybrids during their ontogenetic development.

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EXPLANATION OF PLATE IV

Figs. 1–4. First meiotic metaphase plates of an amphidiploid *N. glauca—N. Langsdorffii* with one lighter and several darker bivalents at this level. The polar ends of the lighter chromosomes (centromeres) are somewhat darker, which suggest that small heterochromatic regions are situated around the centromeres.

Fig. 5. First meiotic anaphase of the same plant with delayed terminalization in a bivalent with two chiasmata (arrow).

Figs. 6 and 7. One of the component chromosomes (upper) of the lighter pair (right) is darker than the other. (The same plant.)

Figs. 8 and 11. Single bivalents of two different P.M.C.'s of the same amphidiploid with differentiated heterochromatic regions turned toward the poles.

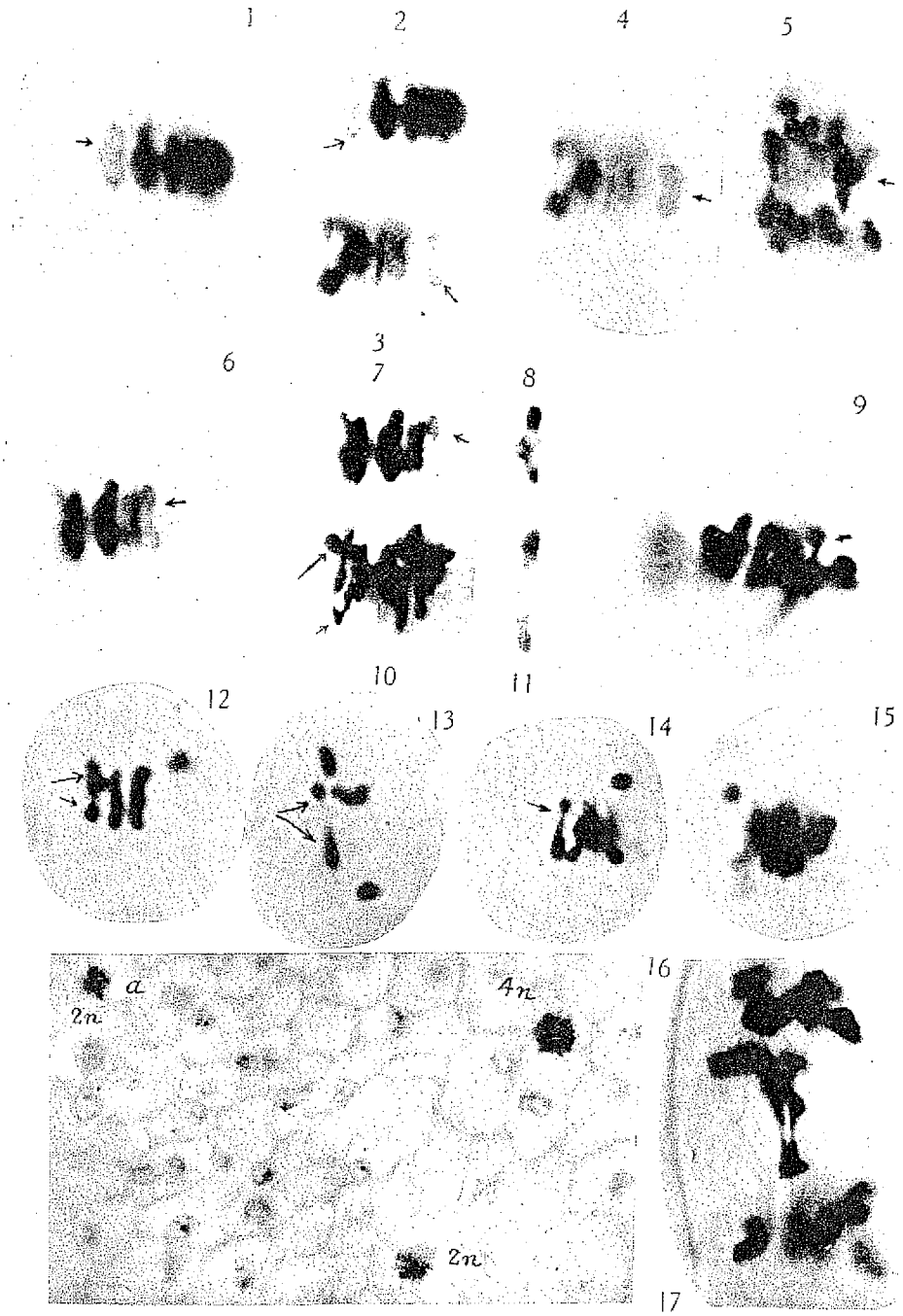
Fig. 9. First meiotic metaphase of the same amphidiploid with heteromorphic bivalent (arrow).

Fig. 10. First meiotic metaphase with a multivalent and a univalent in the same amphidiploid plant.

Figs. 12–15. P.M.C.'s of the F_1 *N. glauca—N. Langsdorffii* with various bivalents and univalents and with heteromorphic bivalent (12–14).

Fig. 16. Microphotography of the tissues of a tumour taken from F_1 *N. glauca—N. Langsdorffii*. Note tetraploid region.

Fig. 17. Delayed separation of a multivalent in an amphidiploid *N. glauca—N. Langsdorffii*.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