DIRECTED HERITABLE VARIATIONS CONDITIONED BY EUPLOID CHROMOSOME ALTERATIONS

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(With Five Text-figures)

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Introduction

Chromosome doubling (autopolyploidy and allopolyploidy) and chromosome reduction (haploidy) are processes which always condition a series of hereditary variations. The euploid chromosome changes, i.e. complete haploidy and polyploidy represent directed variations in a strict sense. Haploids having n chromosomes might produce diploids with $2n$ chromosomes after duplication. When a haploid (n) and a diploid ($2n$) gametes fuse, triploid ($3n$) plants originate. Tetraploids ($4n$) originate by chromosome doubling in diploids or by fusion of two diploid gametes, etc. There is a series of characters that varies parallel into definite directions with the euploid chromosome changes: n, $2n$, $3n$, $4n$, $5n$, $6n$, etc. Good examples of such variations are: (1) Gradual increase of the nuclear and cell sizes as a sequence of the euploid chromosome increase (duplications) and vice versa—a decrease in the nuclear and cell sizes as a sequence of the euploid reduction of the chromosomes. (2) Gradual increase of the breadth of the leaves in respect to their length as a sequence of the euploid increase of the chromosomes, and vice versa—a decrease in the breadth of the leaves in respect to their length as a sequence of the

euploid reduction of the chromosomes (haploidy). (3) An increase in the size of the seeds as a sequence of the chromosome duplication, etc.

Chromosome doubling usually leads to a prolongation of the vegetation period, while chromosome reduction tends to a somewhat earlier flowering. Such regularities obey a series of characters, but there are also characters that change at random, depending on the plant in which the chromosome duplication has taken place. The size of the flowers, and the size of the plants, for example, might increase or decrease after chromosome doubling, depending on the plant in which duplication occurs. I shall consider here chiefly the above-mentioned three characters that change into a definite direction.

Methods and material

Ten years ago Jørgensen (1928) induced chromosome doubling in the genus *Solanum* by decapitation and proposed this method for production of polyploids in other plants. This method is a simplified variation of Winkler's method (1916), who proposed first grafting and then decapitation for inducing polyploidy. At the present time we do not know exactly what kind of processes proceed in the wounded tissue that lead to chromosome doubling. The assumption that wound hormones are the responsible substances for the duplication could satisfy the biologists many years ago, but at the present time it does not suffice to interpret causally step by step the whole process.

Just at the time when the decapitation method gained great popularity, Randolph (1932) reported that polyploid plants can be produced by high temperature. Randolph (1932) and later Dorsey (1936) and others exposed selfed or crossed plants at high temperature (40–45° C.) at the time when it is supposed that the first cleavage of the fertilized egg takes place. This method seems to work satisfactorily in Gramineae in which the decapitation method cannot be applied successfully.

Except these two methods, polyploid forms have been produced in various ways: by X-rays (Ichijima in rice), by *Bacterium tumefaciens* (Kostoff & Kendall, 1932, 1934), by centrifuging (Kostoff, 1937, 1938), etc., shortly, all agents that interfere with the meiotic and mitotic processes. Hybrids, especially intergeneric and most of the interspecific ones as well as heteroploid plants, have irregular meiosis, form occasionally unreduced gametes, and sometimes give rise to polyploid forms. Hybrids that form large numbers of unreduced gametes (*Nicotiana rustica* × *N. paniculata*) give rise more frequently to polyploid, especially amphidiploid plants. Unreduced gametes, formed by F_1 hybrids in a

limited number, were successfully used by the author (1934) in producing polyploids by crossing F_1 (AB) first to the one of the parents (A) and then to the other (B). By this method the following amphidiploids were produced: [(*N. rustica* × *paniculata*) × *rustica*] × *paniculata*; [(*N. glauca* × *Langsdorffii*) × *Langsdorffii*] × *glauca*; (*N. sylvestris* × *tomentosiformis*) × *sylvestris* × *tomentosiformis*; etc.

Finally, a series of allopolyploids were induced apomictically in pollinating F_1 hybrids with pollen from one of the paternal species or by pollen from a third species, as, for example, F_1 (*Triticum Timopheevi* × *Tr. monococcum*) × a turgidoid segregate from the triple cross *Tr. vulgare—turgidum—dicoccum* (Kostoff, 1936) and many others.

Haploid and polyploid forms, used for the studies here reported, were: *Petunia violaceae* $2n$ and $4n$, *Solanum Lycopersicum* $2n$ and $4n$, *Nicotiana glauca* $2n$ and $4n$, *N. Langsdorffii* n and $2n$, *N. sylvestris* n and $2n$, *N. rustica* n and $2n$; F_1 hybrids, amphidiploids and parental forms of *Nicotiana* species, namely, *N. glauca—Langsdorffii*, *N. rustica—paniculata*, *N. rustica—glauca*, *N. rustica—tabacum*, *N. multivalvis—suaveolens*, *Triticum Timopheevi—monococcum*, and *Tr. dicoccum—Haynaldia villosa*. They were obtained by various methods (cf. Kostoff, 1930*a*, 1934, 1938*a*; Kostoff & Kendall, 1930, 1933).

Very recently, Blakeslee & Avery (1937), Nebel & Ruttle (1938) and the author (1938*b*) induced chromosome duplications by colchicine solutions. Another chemical agent that induces similar effects is acenaphthene. Its biological significance was shown by Shmuck (1938), while its influence upon the mitotic processes was studied by the author (1938*b*, 1938*c*) and by Navashin (1938). Its effect upon the procedure of the meiotic processes was studied by the author (Kostoff, 1938*d*, *e*).

Colchicine solutions and sublimated particles from acenaphthene crystals paralyse the activity of the factors that participate in the metaphasal arrangements of the chromosomes at the equator and in the formation of the achromatic figures (spindle). During the mitosis the chromosomes divide but do not separate; thus chromosome doubling takes place. In some cases the chromosomes get spread into the cytoplasm. This takes place especially during the meiosis under the influence of acenaphthene particles. It leads to formation of numerous nuclei with various chromosome numbers. During the cytokinesis numerous microspores are formed with various chromosome numbers, some having more than one nuclei.

The results obtained recently in experimenting with colchicine and acenaphthene showed that at the present time these two chemical agents

are the most promising ones for inducing chromosome duplications. In applying colchicine, I obtained polyploid plants in *Nicotiana Sanderae*; in applying acenaphthene, I obtained polyploids in *Nicotiana longiflora*, *Triticum durum*, *Festuca pratensis*, etc.

Each one of the above-mentioned methods represents a logical outcome of previous works upon the reaction of plant and animal cells and tissues to : (1) woundings, (2) temperature, (3) chemicals, (4) bacteria, etc. In some of the above-mentioned cases chromosome doubling is probably preceded by an increase in the protoplasmic viscosity or the agents act somewhat on the spindle formation, which participates in the process of chromosome separation. In our earlier papers (Kostoff, 1930, 1931; Kostoff & Kendall, 1931, 1932, 1933, 1934; Kendall, 1930) we expressed the opinion that agents increasing the protoplasmic viscosity should act as inductors of chromosome doublings. Recent experiments supported this opinion in many respects. The new trend of the researches should be directed towards the investigation of the chain of processes that proceed from the time of action of the chromosome doubling inductor until the completion of the chromosome doubling in the reactor, and there is no doubt that more effective agents for chromosome doubling can be found after such a kind of investigation.

The methods applied until the present time for chromosome doubling (including those applied in doubling the chromosomes in partially fertile or sterile hybrids) and their perfection by new researches, open a new era in biology, especially in plant biology.

We know at the present time that chromosome doubling in plants is not simply a mechanical process, a "tetraploid" is not simply a "diploid" with twice as many chromosomes, but a new biological system. I am broadly taking up here in this paper only a few characters for the illustration of this statement, namely, the cell size, the leaves; in respect to their shapes, particularly length and breadth, and seed weight, but a series of other characters are also briefly considered. I shall consider here three types of euploid chromosome changes: (1) autotetraploidy, (2) allopolyploidy, and (3) haploidy in tobacco, tomato, *Petunia* and wheat.

The characters studied

(a) *Leaf breadth*

Tetraploid tomato ("Mikado") has shorter but much broader leaves. The variety "Mikado", from which we had obtained tetraploid forms (Kostoff & Kendall, 1934), has composite leaves as any tomato variety,

but its leaflets are much larger than those of the other varieties. In measuring the length and the breadth of the first two (from the petiole) leaflets in diploid and tetraploid tomatoes we obtained the indexes length:breadth for both forms which show definitely that tetraploids

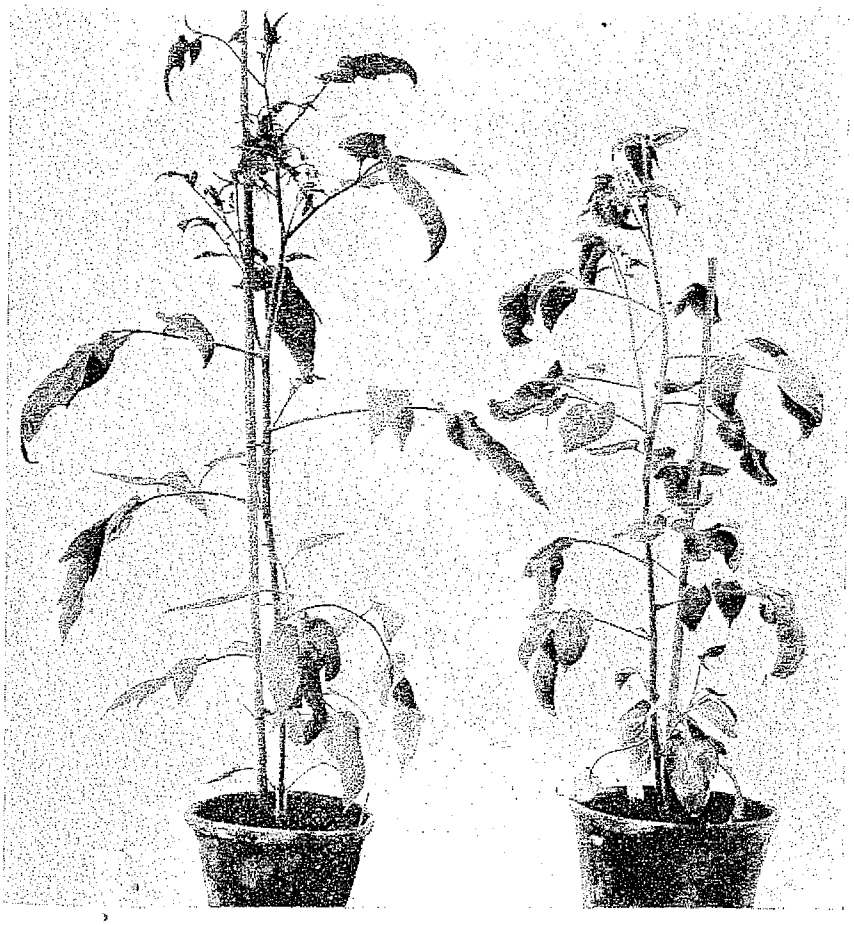

Fig. 1. Diploid (left) and tetraploid (right) tomato ("Mikado").

have broader leaves than diploids. The data are given in Table I. A picture of these plants is given in Fig. 1.

Similar relations were found in measuring the length and breadth in tetraploid and diploid *Nicotiana glauca*. (Plants of these forms were kindly supplied by G. Protassenia, for which I wish to express here my

TABLE I

The leaf index length : breadth in $2n$ *and* $4n$ *tomato "Mikado"*

Forms	Somatic chromosomes	Leaf index length : breadth										n	M	σ
		0·80–0·99	1·00–1·19	1·20–1·39	1·40–1·59	1·60–1·79	1·80–1·99	2·00–2·19	2·20–2·39	2·40–2·59	2·60–2·79			
Diploid	24	—	—	—	1	3	18	33	30	15	2	102	2·17	± 0·23
Tetraploid	48	6	15	27	17	4	2	—	—	—	—	71	1·31	± 0·23

$Md - Mt = 0{\cdot}86$, $m_{diff.} = 0{\cdot}04$ ($Md - Mt = 0{\cdot}86 \pm 0{\cdot}04$).

TABLE II

The leaf index length : breadth in $2n$ *and* $4n$ Nicotiana glauca

Forms	Somatic chromosomes	Leaf index length : breadth							n	M	σ
		1·20–1·29	1·30–1·39	1·40–1·49	1·50–1·59	1·60–1·69	1·70–1·79	1·80–1·89			
Diploid	24	—	—	6	12	10	4	1	33	1·59	± 0·10
Tetraploid	48	8	15	6	—	—	—	—	29	1·34	± 0·07

$Md - Mt = 0{\cdot}25 \pm 0{\cdot}02$.

TABLE III

The leaf index length : breadth of the F_1 *hybrid* Nicotiana glauca ($n = 12$) × Langsdorffii ($n = 9$) *and its amphidiploid* ($2n = 42$)

Forms	Somatic chromosomes	Leaf index length : breadth									n	M	σ
		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			
F_1 hybrid	21	—	—	—	3	18	24	15	4	1	65	1·88	± 0·11
Amphidiploid	42	2	15	24	23	8	1	—	—	—	73	1·58	± 0·11

$Mh - Ma = 0{\cdot}30 \pm 0{\cdot}02$.

TABLE IV

The leaf index length : breadth of the leaves in Nicotiana sylvestris *haploid and diploid plants*

Forms	Somatic chromosomes	Leaf index length : breadth									n	M	σ
		1·85–2·04	2·05–2·24	2·25–2·44	2·45–2·64	2·65–2·84	2·85–3·04	3·05–3·24	3·25–3·44	3·45–3·64			
Haploid	12	—	—	—	—	—	1	6	9	1	17	3·26	± 0·10
Diploid	24	2	8	18	9	2	—	—	—	—	39	2·3	± 0·12

$Mh - Md = 0{\cdot}96 \pm 0{\cdot}03$.

gratitude.) Tetraploid *N. glauca* has also broader leaves (Fig. 2) than the diploid form as shown in Table II.

It should be mentioned here that other strains of *N. glauca* have narrower leaves. I have measured the leaves from *N. glauca* plant vegetatively propagated by cuttings from which Protassenia produced the tetraploid one—and kindly sent me rooted shoots.

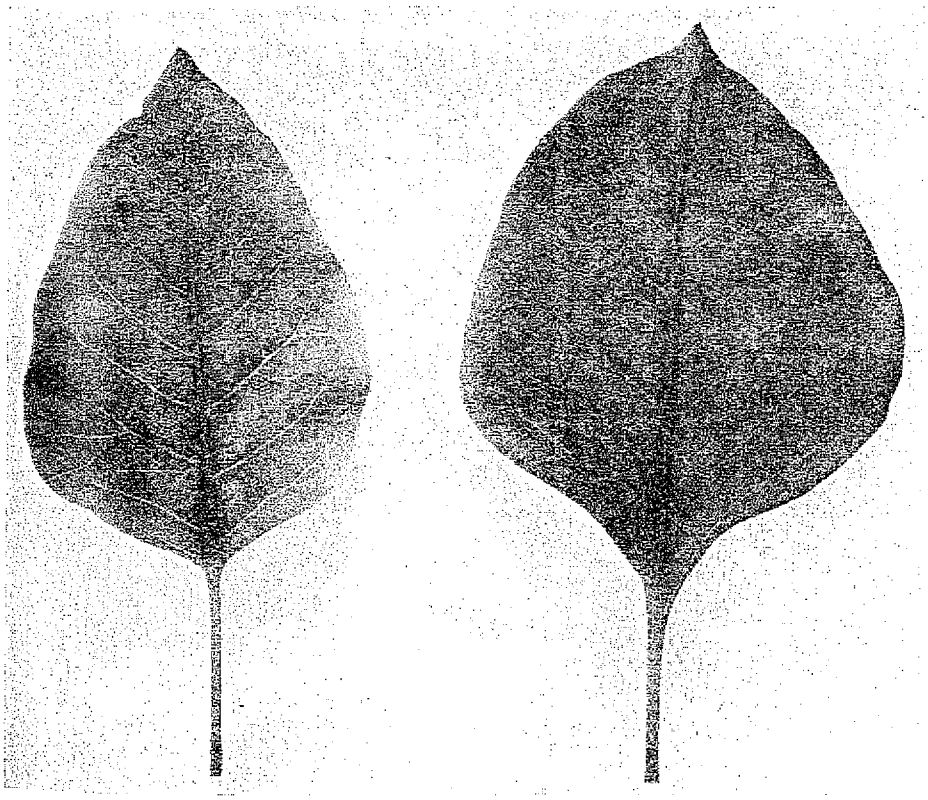

Fig. 2. Leaves from diploid (left) and tetraploid (right) *Nicotiana glauca*.

The tetraploid form was also propagated by cuttings in order to avoid "segregation".

I gave above two examples which show that autotetraploids produced by chromosome doubling in diploids have relatively broader leaves. The increase of the breadth of the leaves in tetraploids in respect to the length is a magnitude that does not vary very greatly, while the length alone is a more variable one. In some tetraploids the length of the leaves increases

somewhat with a significant increase of the breadth, in others the length of the leaves in the tetraploids is not increased, and in a third group of tetraploids the length of the leaves decreases at the same time when an increase in breadth takes place.

Allotetraploid plants produced by chromosome doubling in F_1 hybrids have also broader leaves like the autotetraploids in respect to the diploids.

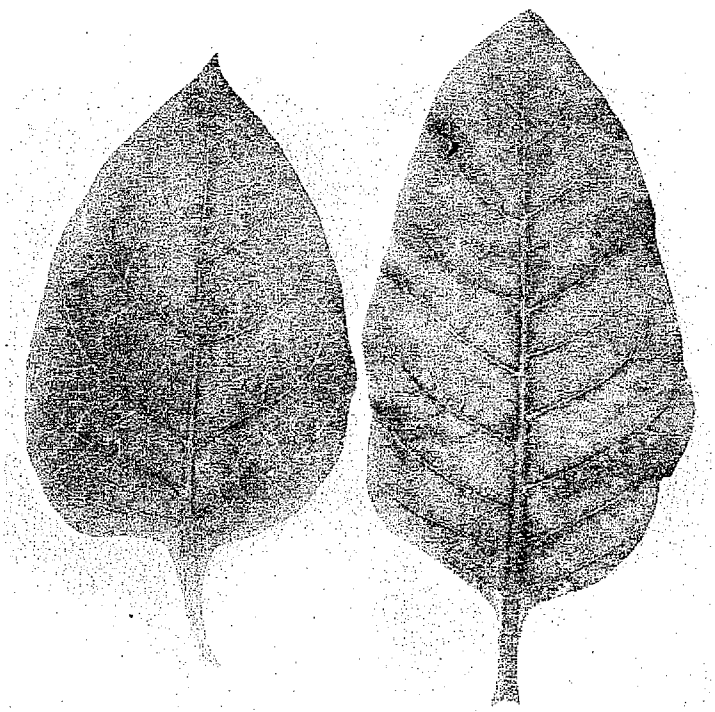

Fig. 3. Leaves from the F_1 hybrid *Nicotiana glauca* × *N. Langsdorffii* (right) and from its amphidiploid (left).

I shall give here in Table III, as an example, the index of the length: breadth of the leaves in the F_1 hybrids *N. glauca* × *Langsdorffii* ($2n=21$), and in the allotetraploid (amphidiploid) *N. glauca—Langsdorffii* ($2n=42$) (Fig. 3).

It should be mentioned here that the amphidiploids: *N. rustica—paniculata*, *N. multivalvis—suaveolens*, *N. rustica—glauca* and *N. rustica—tabacum* have much broader leaves than the F_1 hybrids and smaller indexes length : breadth than their F_1 hybrids. Similarly the amphi-

diploids *Triticum Timopheevi—monococcum* and *Tr. dicoccum—Haynaldia villosa* have broader leaves than their F_1 hybrids.

The above-given examples showed that the chromosome duplication leads to an increase of breadth in the leaves.

The studies on the haploids show, on the other hand, that the reduction of the chromosome number, i.e. haploidation, leads to a considerable

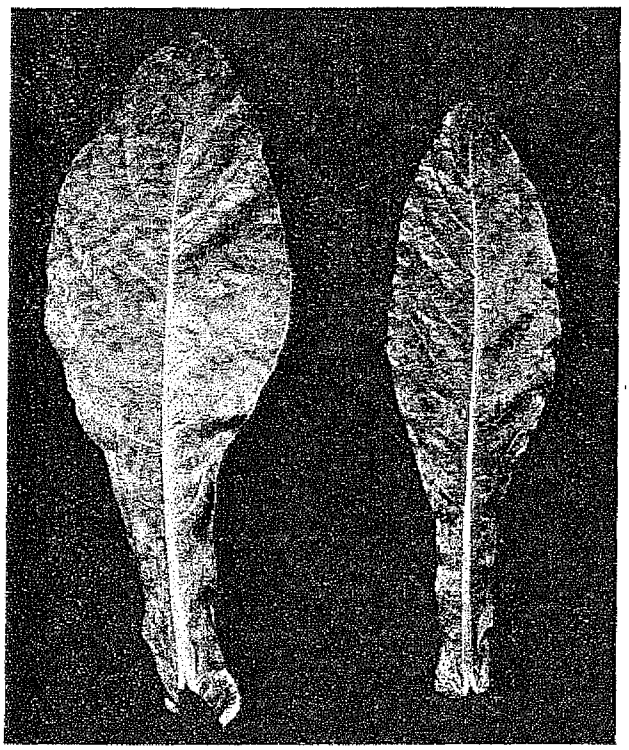

Fig. 4. Leaves from diploid (left) and haploid (right) *Nicotiana sylvestris* plants.

reduction (narrowing) in the breadth of the leaves. *Nicotiana sylvestris* haploid ($n=12$) in comparison with the diploid *N. sylvestris* ($2n=24$) will serve us as an example. In Table IV the index length : breadth of the leaves in the haploid and diploid *N. sylvestris* is given (Fig. 4).

The data given in Table IV show that diploids have broader leaves than haploids. Haploid *N. rustica* had also narrower leaves than the diploid form from which it originated. Diploids produced from the haploid *N. rustica* by selfing the haploid and diploids produced from the

haploid *N. sylvestris* had leaves like the diploids from which the haploids originated. These examples, in addition to those given above, show that chromosome duplication leads to an increase in the breadth of the leaves.

This statement is of great significance from a practical point of view. Some plants, the leaves of which are the commercial product, as in some strains of alfalfa, for example, can be rendered more useful after chromosome doubling. Such plants will have broader leaves. In carrying out such kind of work one should not forget that a too great accumulation of chromosomes affects the vitality of plant organism (Kostoff, 1935). Plants with *ca.* 200 chromosomes are not "giants" but rather "dwarfs" when compared with the species of the same genus.

(b) Cell and nucleus size

In 1905 Boveri showed that the size of the nuclei in echinoderm larvae is dependent upon the number of the chromosomes the nuclei contain. In a later paper he reported that the surface of the nucleus is proportional to the chromosome number and the size of the cell is proportional to both. More recent investigations (Gates, 1909; Tischler, 1921–22; Wettstein, 1924; Karpechenko, 1928; Kostoff, 1934; Kostoff & Kendall, 1934; Sinnott *et al.* 1934, and many others) showed that polyploid plants have larger nuclei and cells no matter that there is not constantly an exact proportional increase of the cell and nuclear size with the chromosome doubling. Haploids have smaller cells (cf. Kostoff, 1938) than diploids (Tables V–VII), while tetraploids have larger cells than diploids (Tables VIII–XV).

I am giving in Table V the diameter of the pollen mother cells of the haploid and diploid *N. sylvestris* during the I late metaphase and I early anaphase. The difference is very striking, the haploid $M = 17{\cdot}47$ and diploid $M = 23{\cdot}83$. The data given in Table VI show that the length of the stomata cells in diploid *N. rustica* ($M = 46{\cdot}7\,\mu$.) is almost twice as large as that of the haploid *N. rustica* ($M = 26{\cdot}4\,\mu$.). In Table VII I am giving the ratio of the linear dimensions and of the volumes of cells in diploids and haploids from various organisms, including those of the haploids and diploids which I have recently studied.

The same tendency, though, in some cases, less striking, can be found in comparing the sizes of the cells in diploids and tetraploids. I am reporting in Table VIII the data obtained in measuring pollen mother cells in tobacco polyploid plants. The data given in this table show that the nuclei increase with the increase of the chromosome number. Cells

TABLE V

Diameter of the pollen mother cells in haploid and diploid Nicotiana sylvestris *during the late I metaphase and early anaphase*

Number of the cells measured ...	Diameter of the pollen mother cells in microns												n	M	σ
	13·5	15·0	16·5	18·0	19·5	21·0	22·5	24·0	25·5	27·0	28·5	30·0			
Forms: Haploid	2	19	75	64	33	5	2	—	—	—	—	—	200	17·4	± 1·56
Diploid	—	—	—	1	5	20	43	57	39	17	8	1	200	23·83	± 2·16

$Md - Mh = 6{\cdot}43 \pm 0{\cdot}19$.

TABLE VI

The length of stomata board cells from the leaves of Nicotiana rustica *diploid and haploid plants in microns*

Forms	Microns													n	M	σ
	23–25	26–28	29–31	32–34	35–37	38–40	41–43	44–46	47–49	50–52	53–55	56–58	59–61			
Haploid	8	24	40	16	3	—	—	—	—	—	—	—	—	91	26·4	± 4·12
Diploid	—	—	—	—	—	9	12	10	9	6	7	3	2	58	46·7	± 5·89

$$\text{Ratio}: \frac{\text{haploid}}{\text{diploid}} = \frac{1}{1{\cdot}4}$$

$Md - M\,h = 20{\cdot}3 \pm 0{\cdot}88$.

also increase with the increase of the chromosome number. Measuring, however, the diameter of the cytoplasm together with the nucleus (MC) and the diameter of the nucleus (MN) and subtracting the latter from

TABLE VII

Ratios between linear dimensions and volume dimensions in haploid and diploid organisms

	Organism	Dimension	Tissue or cells	Haploid	Diploid	Author
(1)	*N. Langsdorffii*	Linear	Root cells	1	1·6	Kostoff, 1929
(2)	*N. Langsdorffii*	Volume	Root cells	1	4	Kostoff, 1929
(3)	*N. sylvestris*	Linear	PMC, I anaphase	1	1·31 (also 1·36)	Kostoff
(4)	*N. sylvestris*	Volume	PMC, I anaphase	1	2·24 (also 2·50)	Kostoff
(5)	*N. rustica*	Linear	Stomata cells	1	1·4	Kostoff
(6)	*N. rustica*	Volume	Stomata cells	1	2·74	Kostoff
(7)	*Crepis capillaris*	Volume	Root cells	1	2·22	Navashin, 1931
(8)	*Drosophila*	Linear	Eye facets	1	1·21	Bridges, 1925
(9)	*Drosophila*	Cubical	Eye facets	1	1·77	Bridges, 1925
(10)	*Uvularia*	Linear	Pollen	1	1·25	Belling, 1925
(11)	*Funaria hygrometrica*	Cell volume	Leaf cells	1	1·8	Wettstein, 1924

TABLE VIII

The diameter of the nuclei, cytoplasm of the cells (somewhat contracted from the fixation), and the cells (the cell walls) in microns

Plants	Nuclei			Cytoplasm with the nuclei			The cells (cell walls)			
	n	MN	σ	n	MC	σ	n	M	σ	$MC-MN$
Amphidiploid (*N. rustica* × *paniculata*), $2n=72$:										
(*a*) Leptotene	100	15·22	1·19	100	23·75	1·82	100	25·59	2·53	8·53
(*b*) Diakinesis	100	14·87	1·62	100	22·62	1·78	100	24·93	2·28	8·75
(*c*) I metaphase*	100	13·45	1·3	100	22·38	1·96	100	24·92	2·27	8·93
(*d*) II metaphase	100	11·03	1·84	100	21·21	1·63	100	25·95	2·53	—
(*e*) Tetrad stage	—	—	—	100	21·57	2·00	100	27·03	3·34	—
F_1 (*N. rustica* × *paniculata*), $2n=36$:										
(*a*) I metaphase	100	10·86	1·03	100	18·86	1·23	100	22·89	1·99	8·00
(*b*) II metaphase	100	8·74	1·6	100	17·25	0·97	100	21·65	1·85	—
(*c*) Tetrad stage	—	—	—	100	17·07	1·2	100	20·91	1·56	—
(*N. rustica* × *paniculata*) × *rustica*, $2n=60$:										
(*a*) Diakinesis	100	13·82	1·29	100	21·07	1·48	100	24·97	2·2	7·25
(*b*) I metaphase	100	12·14	1·18	100	21·06	1·68	100	25·87	2·32	8·2
(*c*) II metaphase	100	9·88	2·3	100	20·57	1·17	100	25·16	2·41	—
(*d*) Tetrad stage	—	—	—	100	18·88	2·04	100	21·00	2·86	—
Amphidiploid (*N. rustica* × *paniculata*), $2n=72$, another plant:										
(*a*) Leptotene	100	14·83	1·13	100	22·74	1·81	100	25·92	2·57	7·91
(*b*) Diakinesis	100	15·75	1·1	100	23·41	1·51	100	27·02	2·31	7·66
(*c*) I metaphase	100	13·87	1·04	100	24·01	2·2	100	27·48	2·84	10·14

* For the column "nuclei" during the metaphases, the diameters of the metaphase plates are given.
MN = average value of the nucleus diameters.
MC = average value of the diameters of the cytoplasm.

the former ($MC-MN$) at various stage of meiosis, I obtained data which show that the distances (d) between nucleus surface and the surface of the cytoplasm do not differ significantly in polyploid plants with various

chromosome numbers. The volume of the cytoplasm, however, increases with the increase of the chromosome numbers (Table IX).

TABLE IX

The volume of the nucleus, cytoplasm with nucleus, cytoplasm alone, cell wall extent in cubic microns

Plants	n	Nucleus	Cytoplasm with nucleus	Cytoplasm alone (without nucleus)	Cell wall extent
(1) *Amphidiploid* (*N. rustica* × *paniculata*), $2n=72$:					
(*a*) Leptotene	100	1693·10	6162·00	4468·90	8667·38
(*b*) Diakinesis	100	1727·70	6089·10	4361·40	8108·59
(*c*) I metaphase*	100	426·63	5866·22	5439·59	8147·35
(2) Another amphidiploid plant:					
(*a*) Leptotene	100	1706·88	6153·89	4447·01	9041·56
(*b*) Diakinesis	100	2044·65	6711·54	4666·89	10326·65
(*c*) I metaphase	100	448·50	7243·61	6795·11	10859·97
(3) (*N. rustica* × *paniculata*) × *rustica*, $2n=60$:					
(*a*) Diakinesis	100	1381·34	4895·21	3513·86	7509·78
(*b*) Metaphase	100	422·86	4888·26	4465·40	9060·82
(4) F_1 (*N. rustica* × *paniculata*), $2n=36$:					
I metaphase	100	277·75	3510·77	3233·02	6284·70

* For the column "nucleus" during the metaphase, the volume of the metaphases was calculated after the formula r^2h, where h is the thickness of the metaphase plate measured from the side (i.e. the extension of the bivalents—side view).

Tetraploids and amphidiploids have larger pollen grains than their diploid forms and F_1 hybrids (Table X). Larger pollen grains (from plants with larger chromosome number) germinate with thicker pollen tubes (Tables XI and XII). Tetraploids have larger somatic cells than diploids. Examples are given in Tables XIII–XV which show that the periblem cells in root tips and stomata cells are larger in tetraploid plants.

(*c*) *Size of seed*

Chromosome doubling affects many other characters of the plant organism. Tetraploid plants have much larger seeds than diploids. Twenty-three seeds of the tetraploid tomato ("Micado") weighed as much as thirty seeds of the diploid form from which the tetraploid was produced. I shall mention here that this difference is not due to the smaller percentage of seeds set by the tetraploid, since the seeds from the diploid plants were taken out from fruits having about as many seeds as those of the tetraploid form. This was obtained by castrating and selfing the flowers of the diploid by very small amounts of pollen. Thirty seeds (obviously normal) from the tetraploid *N. glauca* weighed as much as thirty-nine seeds from the diploid.

TABLE X

Diameter of the pollen grains in microns

Plants	Diameter in microns: 20·02	22·88	25·74	28·60	31·46	34·31	37·18	40·04	42·90	45·76	48·62	51·48	54·34	57·20	60·06	62·92	65·78	n	M	σ
(1) *N. paniculata* ($2n=24$)	—	7	17	39	105	28	3	—	—	—	—	—	—	—	—	—	—	199	30·56	±2·75
(2) *N. rustica* var. *humilis* ($2n=48$)	—	—	—	—	1	25	89	63	22	—	—	—	—	—	—	—	—	200	38·32	±2·46
(3) F_1 (*N. rustica* × *paniculata*) ($2n=36$)	—	8	25	45	50	29	10	3	3	—	7	3	—	8	—	—	—	191	32·75	±7·65
(4) (*N. rustica* × *paniculata*) × *rustica* ($2n=60$)	—	—	—	7	4	32	27	34	17	23	28	10	4	5	2	1	2	196	40·30	±7·20
(5) Amphidiploid (*N. rustica* × *paniculata*) ($2n=72$)	—	—	—	—	—	6	16	35	119	21	1	—	—	—	—	—	—	200	41·96	±2·60
(6) Diploid tomato ($2n=24$)	10	174	17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	201	22·98	±1·04
(7) Tetraploid tomato ($4n=48$)	—	3	47	129	21	—	—	—	—	—	—	—	—	—	—	—	—	200	28·14	±1·75

$M_6 - M_1 = 11{\cdot}40 \pm 0{\cdot}27$; $M_6 - M_2 = 3{\cdot}64 \pm 0{\cdot}25$; $M_7 - M_6 = 5{\cdot}16 \pm 0{\cdot}14$.

Remark. The hybrids (3) F_1 (*N. rustica* × *paniculata*) and (4) (*N. rustica* × *paniculata*) × *rustica* have very variable pollen grains because they have irregular meiosis, being triploids and pentaploids.

TABLE XI

The thickness of the pollen tubes in Nicotiana *and* Petunia *in microns*

Plants	Thickness (breadth) in microns: 5·72	6·43	7·15	7·86	8·58	9·29	10·01	10·72	11·44	12·15	12·87	13·58	14·30	15·01	15·73	16·44	n	M	σ
(1) (*N. rustica* × *paniculata*) amphidiploid ($6n=72$)	—	—	—	—	1	3	3	2	26	6	7	7	24	4	12	4	99	13·14	±1·90
(2) *N. rustica* ($4n=48$)	—	—	—	—	22	16	18	7	30	1	3	3	1	—	—	—	101	10·49	±1·42
(3) *N. paniculata* ($2n=24$)	2	3	8	14	50	16	7	1	2	—	—	—	—	—	—	—	103	8·54	±0·98
(4) *Petunia violacea*, diploid ($2n=14$)	—	—	9	64	3	1	—	—	—	—	—	—	—	—	—	—	77	7·82	±0·20
(5) *Petunia violacea*, tetraploid ($4n=28$)	—	—	—	1	1	36	6	6	—	—	—	—	—	—	—	—	50	9·50	±0·55

$M_1 - M_2 = 2{\cdot}85 \pm 0{\cdot}24$; $M_1 - M_3 = 4{\cdot}60 \pm 0{\cdot}21$; $M_5 - M_4 = 1{\cdot}68 \pm 0{\cdot}08$.

Amphidiploids also have much larger seeds (and grains) than either of the parental species when the difference between the seeds (size and weight) of the parental plants is not very great and larger than the mean value of the seeds of the parental species when the difference between the seeds (size and weight) of the parental species is great (Tables XVI–XVIII).

TABLE XII

The thickness of the pollen tubes in tomatoes in microns

Plants	Thickness in microns											Total		
	4·7	5·2	5·7	6·2	6·7	7·2	7·7	8·2	8·7	9·2	9·7	n	M	σ
Diploid tomato ($2n=24$)	1	81	62	15	3	1	—	—	—	—	—	163	5·52	$\pm 0{\cdot}38$
Tetraploid tomato ($4n=48$)	—	—	—	1	—	42	48	31	37	18	3	180	8·05	$\pm 0{\cdot}69$

$Mt - Md = 2{\cdot}53 \pm 0{\cdot}06$.

TABLE XIII

Tangential (length, L) and radial (breadth, B) dimensions of the periblem cells in the root tips in microns

Plants	At a distance of 250–300 microns from the tip (end)				At a distance of 700–750 microns from the tip (end)			
	n	ML	MB	$\frac{ML+MB}{2}$	n	ML	MB	$\frac{ML+MB}{2}$
Diploid (*Petunia violacea*), $2n=14$	100	16·09	13·5	14·79	10	22·6	21·4	22·00
Tetraploid (*P. violaceae*), $4n=28$	100	21·12	19·90	20·51	10	34·35	30·75	32·55
Diploid tomato, $2n=24$	100	16·35	16·08	16·21	10	24·31	21·9	23·10
Tetraploid tomato, $4n=48$	100	18·42	18·10	18·26	10	25·8	24·49	25·14

M = average value; L = length; B = breadth.

This can be considered as a (third) general rule. It is of great practical significance. In addition to the amphidiploids given in the Table XVI, the amphidiploids, *Triticum vulgare* × *Secale cereale* and *Triticum durum* × *Secale montanum*, form also larger grains than the parental forms.

The weight of the seeds of the amphidiploid *Nicotiana rustica* × *tabacum* is close to that of the parent with the smaller seeds, because some of the seeds of the amphidiploids are "empty" enclosing air. They do not germinate. The size of the seeds and embryos, however, of the amphidiploids are close to those of the parent with larger seeds (Tables XVI–XVIII).

TABLE XIV

The length of the stomata cells in microns

Plants	Length in microns: 25·74	28·60	31·46	34·32	37·18	40·04	42·90	45·76	48·62	51·48	54·34	57·20	60·06	62·92	65·78	68·64	71·50	*n*	*M*	*σ*
(1) *N. rustica* var. *humilis*, $2n=48$	—	—	1	—	2	2	4	20	24	12	18	11	1	1	—	—	—	96	49·85	±5·30
(2) *N. paniculata*, $2n=24$	2	7	20	39	17	14	—	—	—	—	—	—	—	—	—	—	—	99	34·46	±3·40
(3) F_1 (*N. rustica* × *paniculata*), $2n=36$	—	—	—	1	—	3	8	14	27	13	10	15	1	5	3	—	—	100	50·90	±6·24
(4) Amphidiploid (*N. rustica—paniculata*), $2n=72$	—	—	—	—	—	—	—	—	—	—	3	12	15	34	18	15	2	99	63·09	±3·90

$M_4 - M_3 = 12{\cdot}19 \pm 0{\cdot}74$.

TABLE XV

The breadth of the stomata cells in microns

Plants	The breadth in microns: 8·58	11·44	14·30	17·16	20·02	22·88	25·74	28·60	31·46	34·32	37·18	40·04	42·90	*n*	*M*	*σ*
(1) *N. rustica* var. *humilis*, $2n=48$	—	—	—	6	34	27	22	7	—	—	—	—	—	96	22·58	±3·02
(2) *N. paniculata*, $2n=24$	1	11	62	18	5	—	—	—	—	—	—	—	—	97	14·74	±2·07
(3) F_1 (*N. rustica* × *paniculata*), $2n=36$	—	1	5	45	39	10	—	—	—	—	—	—	—	100	18·65	±2·23
(4) (*N. rustica* × *paniculata*) × *paniculata*, $2n=48$	—	—	—	7	20	21	32	10	8	2	—	—	—	100	24·31	±4·03
(5) Amphidiploid (*N. rustica—paniculata*) × *paniculata*, $2n=48$	—	—	1	3	22	30	26	18	—	—	—	—	—	100	23·77	±3·28
(6) (*N. rustica* × *paniculata*) × *rustica*, $2n=60$	—	—	—	2	2	13	18	42	20	3	—	—	—	100	27·68	±3·40
(7) Amphidiploid (*N. rustica—paniculata*), $2n=72$	—	—	—	—	—	5	13	36	17	21	5	2	1	100	30·43	±4·14

$M_7 - M_5 = 11{\cdot}78 \pm 0{\cdot}47$.

(d) Other characters

Polyploidy affects the size of the flowers in various ways. Tetraploids and amphidiploids have most frequently larger flowers than the diploids (tomato, *Petunia*) and F_1 hybrids (*Nicotiana glauca* × *Langsdorffii*, *N. multivalvis* × *suaveolens*), but there are cases when chromosome duplication leads to a decrease in the flower size (amphidiploid *Galeopsis speciosa—pubescens*, Müntzing, 1932). The chromosome doubling affects

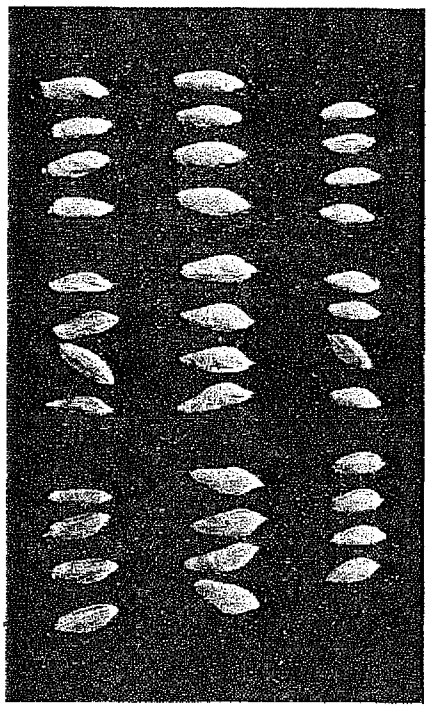

Fig. 5. Seeds: left—seeds from *Triticum Timopheevi*; in the middle—seeds from the amphidiploid *Tr. Timopheevi* × *monococcum* (= *Tr. Timococcum*); right—seeds from *Tr. monococcum*.

in a similar way the size of the plants, i.e. sometimes tetraploids and amphidiploids are larger than diploids and F_1 hybrids, sometimes they are smaller.

Haploids bloom usually earlier than diploids; tetraploids and amphidiploids bloom as a rule (with rare exceptions) later than diploids and F_1 hybrids from which they have originated. A few exceptions are probably due to the unequal environment in which the plants have developed or to

some qualitative genetic differences that arise during the origin of the haploids, tetraploids and amphidiploids.

TABLE XVI

The weight of the seeds of the amphidiploid plants and of their parents in grams

Plants	The weight of 1000 seeds
(1) *Triticum Timopheevi*	34·10
(2) *Tr. monococcum*	24·30
(3) Amphidiploid *Tr. Timopheevi—monococcum*:	
Line *a*	39·20
Line *b*	44·60
Line *c*	45·00
(4) *Tr. dicoccum*	31·80
(5) *Haynaldia villosa*	5·00
(6) Amphidiploid *Triticum dicoccum* × *Haynaldia villosa*	25·40
(7) *Nicotiana rustica* var. *humilis*	0·166
(8) *N. paniculata*	0·035
(9) Amphidiploid *N. rustica* var. *humilis—paniculata*:	
Line *a*	0·162
Line *b*	0·120
(10) *N. Langsdorffii*	0·082
(11) *N. glauca*	0·060
(12) Amphidiploid *N. glauca—Langsdorffii*:	
Line *a*	0·162
Line *b*	0·130
Line *c*	0·124
(13) *Nicotiana rustica texana*	0·252
(14) Amphidiploid *N. rustica texana—glauca*	0·278
(15) *N. tabacum Basma*	0·110
(16) *N. rustica*, a *texana* type	0·236
(17) Amphidiploid *N. rustica* (*texana* type) × *tabacum*:	
Line *a*	0·162*
Line *b*	0·124*
Line *c*	0·118*
(18) *N. multivalvis*	0·230
(19) *N. suaveolens*	0·162
(20) Amphidiploid *N. multivalvis—suaveolens*	0·384

* Some of the seeds are very light, having large empty spaces in the integument filled up with air. Their size is given in Table XVII.

TABLE XVII

Length and breadth of the seeds of Nicotiana rustica, N. tabacum *and their amphidiploid grown at equal environmental conditions*

Plants	Length of the seeds in ocular micrometers $M =$	Breadth of the seeds in ocular micrometers $M =$
N. rustica	43·4	31·1
N. tabacum	33·2	22·6
Their amphidiploid	39·4	31·5

Euploid chromosome alterations affect markedly the thickness of the leaves. Haploids have thinner leaves than diploids, tetraploids have

thicker leaves than diploids. This is obviously due to the smaller cell volume in the haploids and to the larger cell volume in the tetraploids.

The chloroplasts were also measured in the diploid and in the polyploid forms. The data obtained showed that polyploid forms have not larger chloroplasts than the diploid ones (cf. Kostoff & Kendall, 1934). On the contrary, a series of polyploids had somewhat smaller chloroplasts.

TABLE XVIII

Length and breadth of the completely developed embryos from N. rustica, N. tabacum *and their amphidiploid*

	Length in microns			Breadth in microns		
Plants	*n*	*M*	σ	*n*	*M*	σ
(1) *N. rustica*	150	998·5	12·1	150	764·3	9·6
(2) *N. tabacum*	150	684·2	10·5	150	503·7	8·4
(3) Their amphidiploid	20	936·3	15·6	20	718·2	11·7

$Ml_1 - Ml_3 = 62{\cdot}2 \pm 3{\cdot}63$. $Mb_1 - Mb_3 = 46{\cdot}1 \pm 3{\cdot}63$.
$Ml_3 - Ml_2 = 252{\cdot}1 \pm 3{\cdot}59$. $Mb_3 - Mb_2 = 214{\cdot}5 \pm 2{\cdot}70$.

The data obtained from these measurements will be published elsewhere (Kostoff & Orlov). The chloroplasts seem to be more numerous (per cell) in the tetraploid forms than in the diploid.

Some tetraploids are more frost resistant than the diploids. Tetraploid tomato ("Micado") resisted $-5°$ C. during 10 hr., while the diploid forms were killed in 2 hr. at $-1°$ C.

CONCLUSION

The types of realizations of the characters in haploids, diploids, and polyploids show definitely that plants produced by chromosome duplications (polyploidy) and chromosome reductions (haploidy) represent new biological systems in which the quantitative differences in the chromosome numbers condition hereditary changes into definite directions. We have good reasons to expect that further differentiations in these new biological systems should not necessarily proceed in the way they do proceed in the original diploid forms.

In this short paper I have not discussed the role of the polyploidy in evolution, therefore I shall recall here the extensive paper by Müntzing (1936) upon this problem. But, considering the importance of the polyploidy from a phylogenetic and an agricultural point of view at the time when numerous workers apply most promising methods for producing polyploid plants, I shall point out very briefly here the viability of polyploid species. The chromosome numbers determined by various investigators in about 2500 species will give us an idea about the survival

of the polyploid plants in nature. The plants considered have from 3 to 100 gametic chromosomes. Almost all of the species studied are included in this range (3–100). About 400 species out of 2500 have 12 gametic chromosomes, about 350 have 8, about 250 have 7, and about 180 have 9 gametic chromosomes. About 50 species of all studied cytologically (2500) have 40 and more than 40 chromosomes (up to 100) (cf. Fernandes, 1931; Darlington, 1937). It should also be mentioned here that some of the plants having 12 and even 8 chromosomes have been considered as polyploid species. The above data show that a large increase of chromosome numbers affects the vitality and the survival of the plants. This was also shown experimentally (Kostoff, 1935). Hence if we desire to produce polyploid plants without affecting their vitality, species should be used for the purpose with relatively small chromosome numbers.

Summary

1. Decapitation (wounding) method, high temperature, poisons secreted by parasites, etc., induce polyploidy. These methods are not, however, as promising as the treatments with colchicine solutions and with acenaphthene sublimated particles in attempting to produce polyploid forms. Unreduced gametes from species hybrids are also a source for polyploidy.

2. The size of the nuclei, the amount of the cytoplasm, the amount of nucleolus substances, and the cell dimensions increase with the euploid increase of the chromosome numbers (polyploidy) and decrease with the euploid reduction (haploidy) of the chromosomes. The distance between the nuclear surface and the cell wall is a magnitude, which is not significantly influenced by the euploid chromosome changes.

3. The breadth of the leaves of the plants increases in respect to the length when an euploid increase of the chromosomes takes place. It decreases, when an euploid decrease of the chromosomes (haploidy) takes place.

4. The thickness of the leaves increases with the euploid increases of the chromosomes.

5. The size and the weight of the seeds (grains) increases absolutely or relatively when an euploid increase of the chromosomes takes place.

6. The vegetation period (from seed germination until florescence) of the plants usually increases with the euploid increase of the chromosome number.

7. Tetraploid tomato was more cold resistant than its diploid parent.

8. Size of the plant and of the flowers might increase or decrease significantly or insignificantly when an euploid increase of the chromosomes takes place. Haploids, however, have usually smaller flowers and are smaller in size.

9. The size of the plastids usually does not change when an euploid chromosome alteration takes place. In some cases tetraploid forms have somewhat smaller plastids than the diploids.

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