

Adaptive Isochromosomes in *Nicandra*

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With ten Figures in the Text

I. THE SPECIES

NICANDRA PHYSALOIDES is the solitary species of its genus or even tribe. Like some of its nearest relatives in *Nicotiana* it is a native of Peru and Chile. Like them, too, it has become nearly cosmopolitan in cultivation in the last 200 years. It is widely naturalized in the tropics. It was introduced into gardens in this country in 1759 and is still a favourite botanic garden plant. In cultivation and as an escape four completely inter-fertile 'varieties', or rather variants, can be recognized and we are indebted for them to the following sources:

- (i) *typica*, corresponding to the figure in Curtis's 'Botanical Magazine' (No. 2458): from the Botanic Gardens of Kew (4 plants) and of Montreal (30 plants); and collected for us by Dr. S. C. Harland near Lima (6 plants).
- (ii) *violacea*, having stronger pigmentation of stem, petiole, calyx, leaf-hairs, and seeds than *typica* but intergrading polygenically with it; erroneously described by Lemoine (1906) as a distinct species: from Kew Gardens (10 plants).
- (iii) *immaculata*, lacking the corolla spot and the leaf-hair and cotyledon pigmentation; a recessive single-gene variant, heterozygotes selfed having given us 307 normal to 100 spotless (cf. Dahlgren, 1924): collected in Malabar and from Kew Gardens (22 plants).
- (iv) *alba*, with white instead of blue flowers probably also a recessive single-gene variant: from Cambridge Botanic Gardens (6 plants).

The first three variants were also found as escapes from earlier cultivation at Merton.

2. CHROMOSOME TYPES

We can confirm the original chromosome count (Janaki-Ammal, 1932). All the 68 plants of types (i), (iii), and (iv) examined in 1941 had 20 chromosomes. [Annals of Botany, N.S. Vol. IX, No. 35, July, 1945.]

somes in root-tips and flowers.¹ Of the 10 pairs at meiosis, however, one always consists of two isochromosomes. Each of these has two arms with nucleolar constrictions and evidently identical since they are capable of pairing with one another at meiosis. The combination is therefore genetically quadri-valent and the species has the unique property of being regularly tetrasomic for a part of its chromosomes and disomic for the rest.

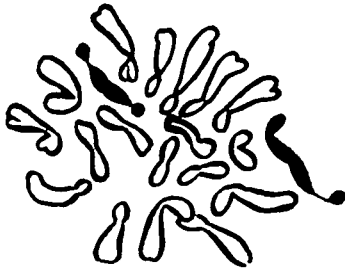


FIG. 1. Root-tip mitosis in normal plant with two isochromosomes (2BD Flemming, gentian violet). ($\times 6,000$.)

The ordinary disomic chromosomes, which we may call autosomes, include yet another pair of nucleolar chromosomes so that there must be in the whole complement of the species six nucleolar organizers.

In resting nuclei of the roots about 20 large pieces of heterochromatin always appear and in the pollen grains about 10. These prochromosomes presumably correspond to the centric segments indifferently of all the chromosomes and the isochromosomes cannot be certainly distinguished. They may, however, be entirely heterochromatic. The premeiotic resting nucleus in the anthers shows no heterochromatin.

At meiosis the two isochromosomes, as we shall see, fail to pair with one another in some pollen mother cells and some of these give rise to pollen grains which lack an isochromosome. Such grains probably do not develop and have not been seen in mitosis. Nevertheless over 90 per cent. of the ripe pollen was good in several individuals examined.

In the embryo-sac similar loss occurs (Fig. 4) and we might expect 9-chromosome egg cells to be formed which, being fertilized, would give a proportion of 19-chromosome progeny. What happens to these deficient embryos? The answer to this question was provided by the germination at Merton in 1941 of seed of *violacea* type which had lain in the ground since 1913. Of 8 plants examined only 2 were normal. The remaining 6 were of the missing type with only one isochromosome ($2n = 19$). Turning to a semi-naturalized population at Kew we again found deficient plants, 3 out of 10.

The deficient seedlings did not owe their deficiency to the age of their seed. Six plants raised from packeted 1930 seed were all normal. Their selective appearance was evidently due to their delayed germination without which indeed the strain would have died out at Merton. To test this explanation a germination test was applied. The 68 seedlings originally examined had all been samples selected (as must happen in pot culture even more regularly than in field culture) for rapid germination. Selfed seedlings of one of the 19-chromosome plants were pricked out in batches according to order of germination and one set was further recorded for order of flowering

¹ Pollen mother cells were smeared with acetic lacmoid containing a trace of Bismarck brown (Darlington and La Cour, 1942). Pollen grains were smeared in half-strength iron aceto-carmin.

(Table I). Our expectation was realized. All the early germination was of normal plants; only later did the deficient appear.

Strains vary widely in seed size. Within strains it is inversely proportional to seed number per capsule which varies in normal plants from 400 to 1,000.

TABLE I A
*Selfed Progeny of a Plant with 19 Chromosomes, type violacea,
with 55.7 per cent. of Full Pollen Grains*

Order of germination shown by pricking out; sown 18/2/42. Number of plants counted shown in brackets

Batch pricked out.	Full pollen grains.		
	>90%	50-90%	0-50%
I. 15 days	16(12)	0	0
II. 25 days	15(15)	4(4)	0
III. 32 days	4(2)	2	5 ¹
	2n = 20	2n = 19	— ¹

¹ Plants too stunted to examine.

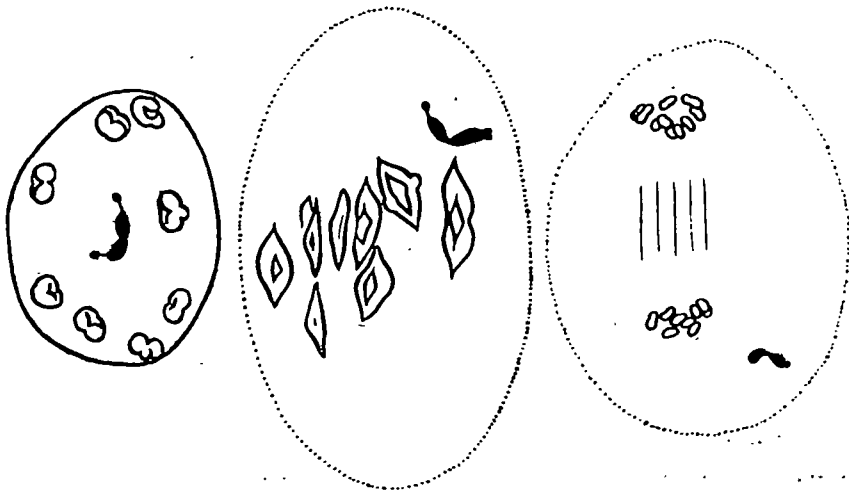


FIG. 2. Diakinesis, first metaphase and anaphase in pollen mother cells of a diploid with one isochromosome (in black). (× 2,200.)

Within capsules seed size is highly uniform. As expected therefore it provided no means of distinction between 19- and 20-chromosome embryos.

The 19-chromosome plants, though often dwarf (Table I B), were not regularly distinguishable from normals in external form. In pollen and egg fertility of course they suffer. Their proportion of full pollen varies from 52 to 56 per cent. Thus half the grains, lacking an isochromosome, die, some of them doubtless too early to figure in the count. One plant recorded as having 19 chromosomes gave 79 per cent. germination, but this must have been due

to the restoration of 20 chromosomes in one anther or follicle by non-disjunction at mitosis.

TABLE I B

Selfed Progeny as in Table I A

Order of flowering; sown 19/12/41

Batch.	$2n = 20$	$2n = 19$
Early germination, <i>normals</i>	0-18	19, 20
Late germination, <i>normals</i>	24, 25	22, 23
<i>dwarfs</i>	—	21, 26, 27

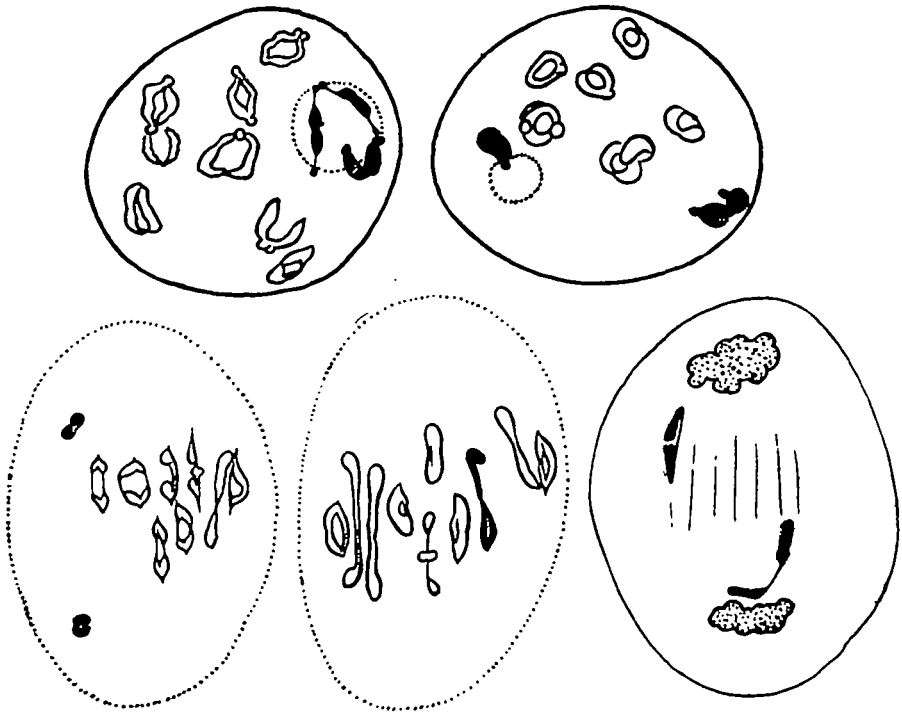


FIG. 3. Diakinesis to first telophase in a diploid with two isochromosomes. The nucleolar autosome pair is hatched. The lagging isochromosomes show attempted misdivision at anaphase. ($\times 2,200$.)

3. THE PRODUCTION OF HAPLOIDS AND POLYPOIDS

Normal diploids set three capsules of seed with pollen of *Hyoscyamus niger*, and one each with garden Petunia and *Nicotiana glauca* (all diploids). The seeds were uniformly small, 33 from the first (11 per capsule), 6 from the second, and 20 from the third cross. They developed, however, into normal maternal diploids. It seems likely that haploid parthenogenesis was followed by mitotic recovery as frequently happens in other solanaceous plants when crossed with foreign pollen.

Tetraploids were produced by treating the selfed seedlings of 20-chromosome plants with a 0.2 per cent. aqueous solution of colchicine. A drop of the solution was applied between the two cotyledons. Fifteen out of 33 proved to be tetraploid. Even higher proportions were obtained by wetting the axillary buds after amputation of the older plants.

The 15 plants had the characteristic stouter growth and larger flowers of tetraploids. They could be critically distinguished from diploids by the folding of the corolla in the bud. The ovary and stamens are disproportionately

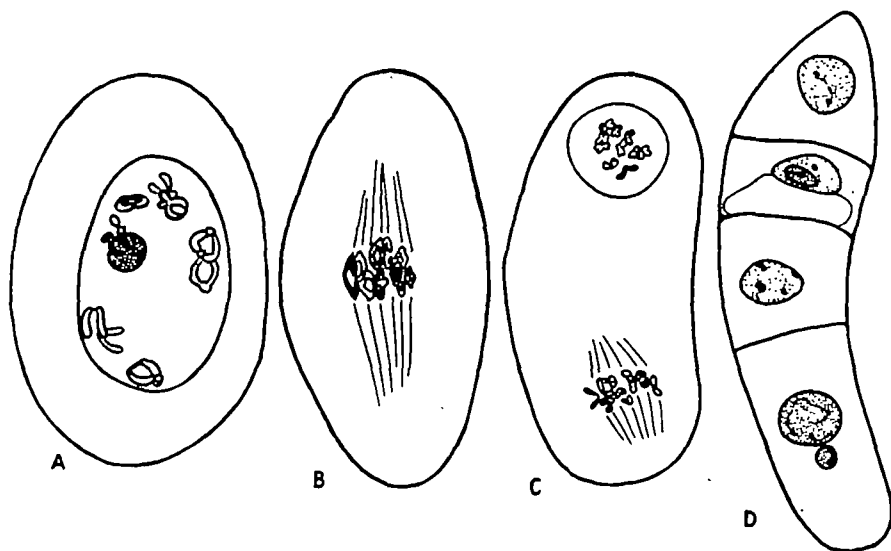


FIG. 4. A—D Diakinesis to second telophase in the embryo-sac mother cell of a diploid with two isochromosomes. An isochromosome has been left out of the main embryo-sac nucleus in D. ($\times 2,200$.)

increased in size so that the corolla is not large enough to cover them completely.

As usual in autotetraploids, the seeds and full pollen grains were twice the volume of those of diploids. As usual also, the pollen and seed fertility was reduced owing to the irregular segregation of quadrivalents at meiosis. Four plants had 52, 58, 67, and 68 per cent. of full pollen grains at maturity. A proportion of giant unreduced pollen grains are formed.

The seed of tetraploids is very variable in size but it begins to germinate more quickly than that of diploids and the selfed progeny is highly uniform in appearance. Eighteen plants examined from early and late germinations all had 40 chromosomes including 4 isochromosomes. About 6 per cent. of seeds, however, fail to throw off the testa and these may have been the ones containing 3 or 5 isochromosomes.

Diploids crossed with tetraploid pollen gave a few apparently good seeds which failed to germinate. The reciprocal cross gave seed of which only

tetraploids germinated. Evidently the tetraploid style had selected unreduced pollen from the diploid (cf. section 6), as it frequently does in such crosses.

Among the selfed progeny of a diploid, 20-chromosome, *immaculata* from Kew, such unreduced or binucleate pollen gave rise to one triploid. This plant resembled a tetraploid rather than a diploid in external appearance. At meiosis it proved to be partially asynaptic in the pollen mother cells. Less than 5 per cent. of its pollen was full and it was totally seed sterile.

Finding the failure of meiosis in a triploid is not just a coincidence. The

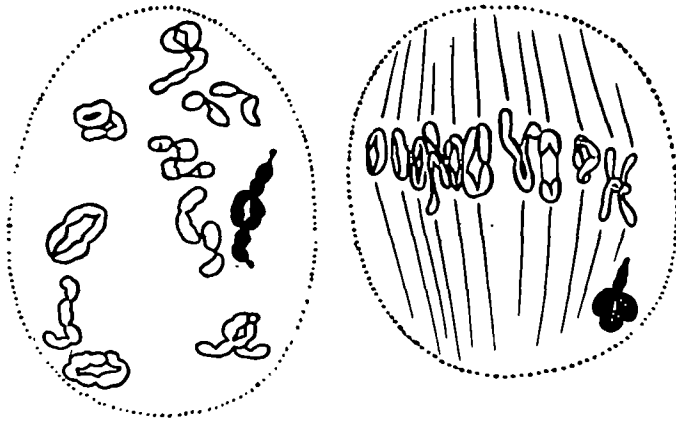


FIG. 5. Diakinesis and first metaphase in the pollen mother cells of a tetraploid with four isochromosomes. ($\times 2,200$.)

combination is favoured in two ways. First, failure of meiosis permits parthenogenesis and is therefore a condition of reproduction in any triploid. This is the case in nature with triploid forms of *Allium amplexans* (Levan, 1940). Secondly, failure of meiosis is likely to be inherited from any parent which itself, as a result of such failure, bore unreduced pollen and hence begot triploid progeny. This is the sequence of events revealed in experiments with *Capsicum* (Ramanujam and Pal, 1940). Our triploid suggests the same history.

4. THE AUTOSOMES AT MEIOSIS

In the diploids with 19 and 20 chromosomes, the autosomes regularly form 9 bivalents with 1 or 2 chiasmata, usually terminalized at metaphase (Figs. 2-4).

In the tetraploids of the second colchicine, or C_2 , generation the frequency and distribution of chiasmata which were recorded were not significantly different from those in the diploid (Table II). Five cells had 42 out of the potential 45 quadrivalents and these were usually of the ring or chain type expected with a low chiasma frequency (Fig. 5).

TABLE II
Chiasmata in Autosomes

Plant.	Cells.	Chrs.	Xta.	X-frequency.
$2x + 1iso$	5	45	79	1.76
$2x + 2iso's$	5	45	80	1.77
$4x + 4iso's$	5	90	164	1.82

5. ISOCHROMOSOMES AT MEIOSIS

Chiasmata in the isochromosomes are either terminal, as in the autosomes, or they lie next to the nucleolar constriction. The centromeres of the isochromosomes, however, must be weaker for, both as bivalents and as quadrivalents, they lag in first metaphase congression and in first anaphase separation. Even at mitosis in root-tips, anthers, and ovaries similar lagging occurs.

At diakinesis the nucleolar constrictions of the isochromosomes are very marked and they even survive at metaphase clearly enough to serve for recognition. A second sign, however, is even more valuable. Failing to congress and lying off the plate, like the sex chromosomes of mammals, they can be readily found and recorded in every cell. It is then seen that the opportunity of

TABLE III

Pairing and Chiasma Formation in Isochromosomes according to Univalent or Bivalent Configuration in 56 P.M.C. of the Normal Diploid ($2x+2iso$'s)

Type of configuration.	I.	II.	Total.
No. of configurations	52	30	82
No. of chromosomes	52	60	112
Xta { within iso's	47	—	47
{ between iso's	—	28	28
{ multiples	—	12	12
TOTAL Xta	47	40	87
Xta p. chromosome	0.90	0.67	0.78

N.B. Chiasma frequency is higher with purely inside pairing than with both inside and outside pairing.

TABLE IV

Pairing and Chiasma formation of Isochromosomes according to Configuration in 50 Cells of the Tetraploid (cf. Fig. 6)

Type of configuration.	I.	II.	III.	IV.	Total.
Nos. of configurations	56	21	26	6	—
Nos. of chromosomes	56	42	78	24	200
Xta { within iso's	51	—	2	2	55
{ between iso's	—	32	61	25	118
{ multiples	—	4	24	5	33
TOTAL Xta	51	36	87	32	206
Xta p. chromosome	0.91	0.86	1.12	1.33	1.03

TABLE V

Summary of the Pairing of different Numbers of Isochromosomes at Meiosis

Types.	$2x+1 = 19$	$2x+2 = 20$	$4x+4 = 40$
Cells	70	56	50
Chromosomes	70	112	200
Xta { within iso's	56	47	55
{ between iso's	—	28	118
{ multiples	—	12	33
TOTAL Xta	56	87	206

TABLE VI

Summary of Chiasma Frequencies in Isochromosomes

Ploidy.	Iso's.	Cells with diff. Nos. of Xta.						Total Xta.	Total Iso's.	Xta p. Chr.
		0	1	2	3	4	5			
2x	1	14	56	—	—	—	—	56	70	0.80
2x	2	1	25	28	2	—	—	87	112	0.78
3x	3	—	—	10	12	13	7	143	126	1.13
4x	4	—	—	—	12	22	14	206	200	1.03

multiple chiasma formation raises the chiasma frequency in polyploids as compared with diploids (Table VI).

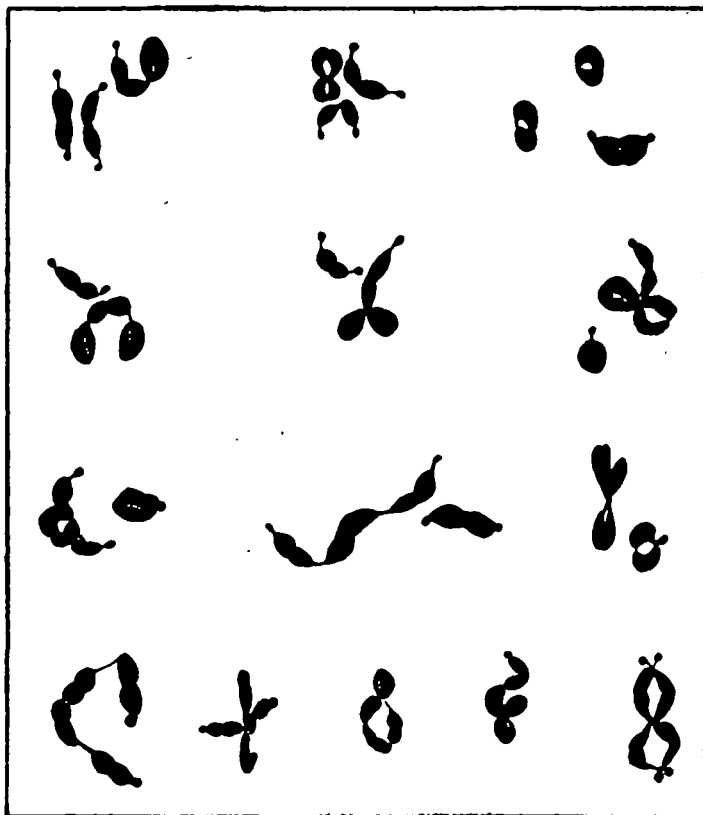


FIG. 6. Configurations of isochromosomes in the tetraploid. ($\times 2,800$.)

The single isochromosome in 19-chromosome plants usually forms a chiasma with itself. This *inside* pairing still has an advantage over *outside* pairing when there are several isochromosomes. Thus the proportions of one to the other expected and found (Tables III, IV, and V) are as follows:

Type.	Observed rates.	Expected.	Advantage factor.
2x + 2iso's	47:28 or 1.68	$\frac{1}{2}$ or 0.50	3.4
4x + 4iso's	55:118 or 0.47	$\frac{1}{4}$ or 0.17	2.8

The position advantage is, as it should be, slightly less in the tetraploid since the chances of outside pairing must increase with the number of iso-

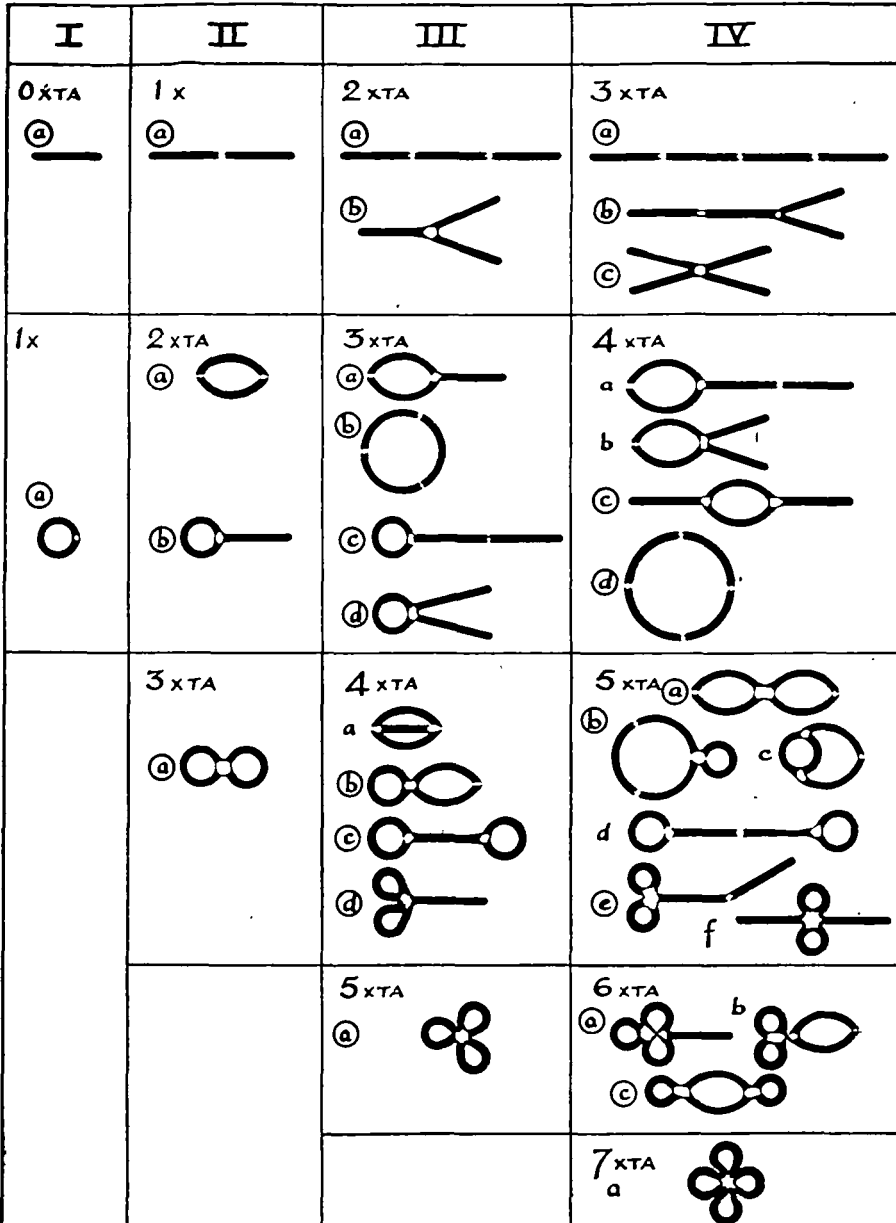


FIG. 7. Diagram showing possible and observed (with letters ringed) configurations of isochromosomes in the tetraploid, according to the numbers of chromosomes and of chiasmata.

chromosomes. This advantage operates against the formation of high configurations, and we find that the trivalent isochromosome is still the most

frequent type even in the tetraploid (Table IV). But its most striking effect appears, as we shall see, only with partial asynapsis.

6. DIFFERENTIAL ASYNAPSIS

Pairing partly fails at meiosis in the pollen mother cells of our spontaneous triploid. This failure provides a critical test of certain conditions of pairing since the two kinds of chromosome differ in regard to these conditions.

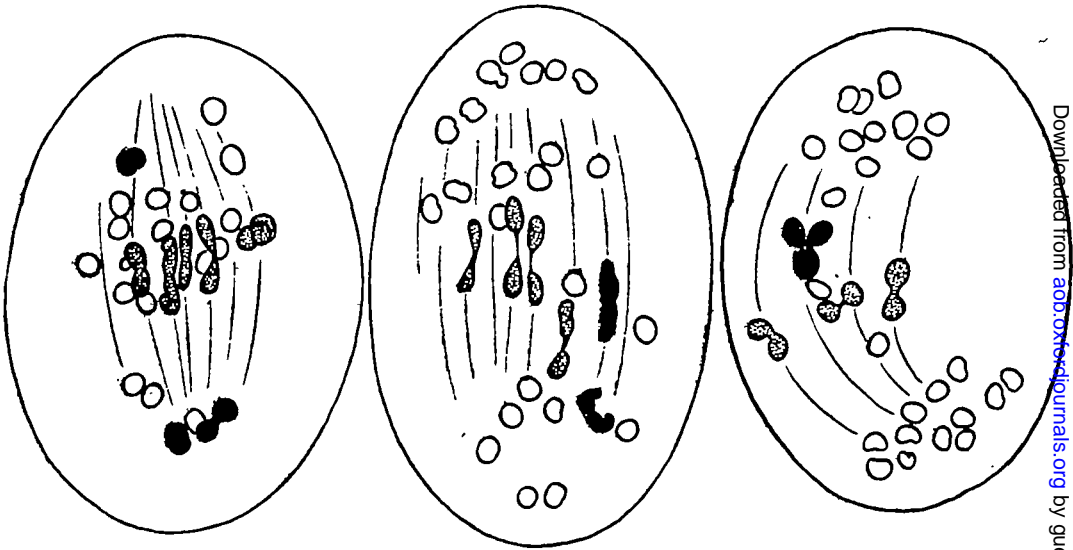


FIG. 8. Abortive first metaphase-anaphase in the triploid with partial asynapsis of autosomes and normal pairing of isochromosomes. ($\times 2,200$.)

The autosomes have to move in order to pair while the isochromosomes have their pairing arms already attached. The reaction of the two types is in fact sharply contrasted. The autosomes, instead of 8 or 9 trivalents, form 3, 4, or 5 bivalents and a rare trivalent. The isochromosomes, on the other hand, have their chiasma frequency unreduced. Rather is it slightly higher in this triploid than in any normal diploid or tetraploid, a property we associate with the trivalent being the most frequent isochromosome configuration even in the tetraploid (Fig. 8 and Table IV).

Asynapsis in this plant is therefore to be attributed to the autosomes having too little time to pair. Prophase is insufficiently precocious and the time limit interrupts pairing before most of the autosomes have come together, although not before the isochromosomes, with their internal advantage of position, have fully united (cf. Frankel et al., 1940). The validity of this explanation could be tested in diploid maize where isochromosomes could be brought together with asynapsis.

7. MITOSIS IN THE POLLEN

Equally in 19- and in 20-chromosome plants the only pollen grain mitoses seen are those with 10 chromosomes (Fig. 9). Evidently pollen lacking the isochromosome dies. Indeed some of it must do so very early since the proportion of empty pollen at this time in the 19-chromosome plants is less than 50 per cent. (Table I and section 2).

Two kinds of abnormalities were found, however, in the pollen of diploids. The first was in an *immaculata* plant from Kew. It had, in different anthers, 20 to 34 per cent. of giant pollen grains. These had been formed owing to the failure of the second division wall after meiosis, as in *Saccharum* (Janaki-Ammal, 1941) and, for both divisions, in the pear *Beurré Bedford* (Thomas unpubl.). Such pollen would be able to produce an effectively diploid gamete for the fertilization of the egg together with a haploid gamete for the fertilization of the endosperm. Thus an apparent obstacle to the direct production of triploids from the crossing of diploids and tetraploids might be overcome (section 3).

The second kind of abnormality was in a *violacea* from Merton. In an anther sample with 43 per cent. of post-mitotic grains and 14 per cent. of mitotic, i.e. in the middle of the mitotic phase, 5 of the 15 mitoses observed were second mitoses of both nuclei (Fig. 10). The pollen was not giant and could not therefore have been originally binucleate. These mitoses were thus supernumerary. Their occurrence apparently goes with a lack of differentiation of vegetative and generative nuclei. A similar situation was occasionally found in *Sorghum*, where it was similarly associated with an over-hasty 'polymitosis' and with the presence of supernumerary chromosomes (Darlington and Thomas, 1941).

TABLE VII

Chromosome Numbers at Mitosis in Pollen Grains of violacea Tetraploids (4x + 4iso's) from Samples differing in the Phase of Development

Plant.	Binucleate Grains.	Chromosome Numbers.			Total.
		18	19	20	
$K_{1^2}a_2$	28%	—	6	16	22
$K_{1^2}a_1$	28%	1	7	12	20
$K_{1^2}a_1$	83%	5	2	4	11

In the tetraploids there is a range of chromosome numbers which depends on the presence of 17 or 18 autosomes and 1 or 2 isochromosomes. In order to find out whether a deficiency of either type was deleterious we compared the frequencies of deficiencies at different mitotic phases (cf. Darlington and La Cour, 1945). The observations showed that the defective pollen, like the defective seeds of the diploid, developed more slowly than the normal (Table VII).

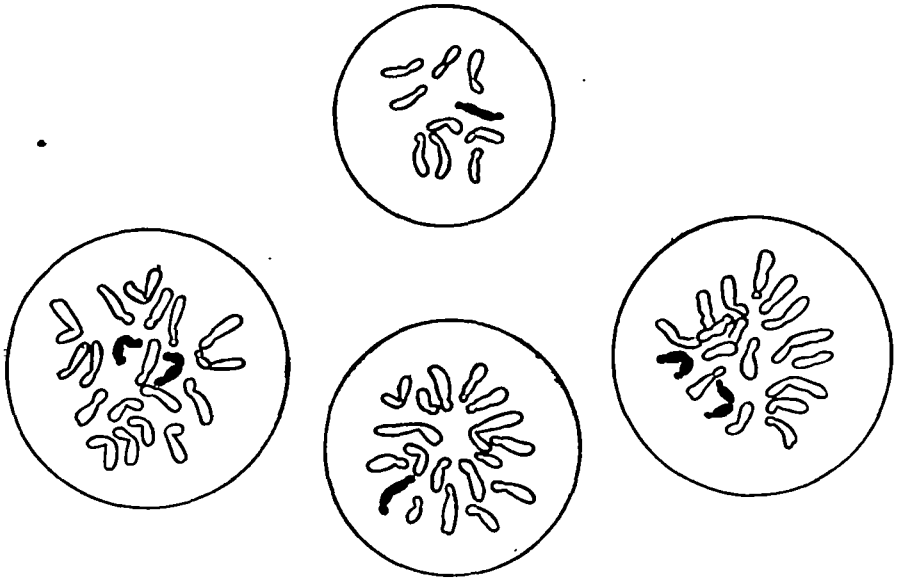


FIG. 9. Pollen grain mitoses of a diploid (above) and of a tetraploid (below) with different numbers of isochromosomes and autosomes. ($\times 2,200$.)

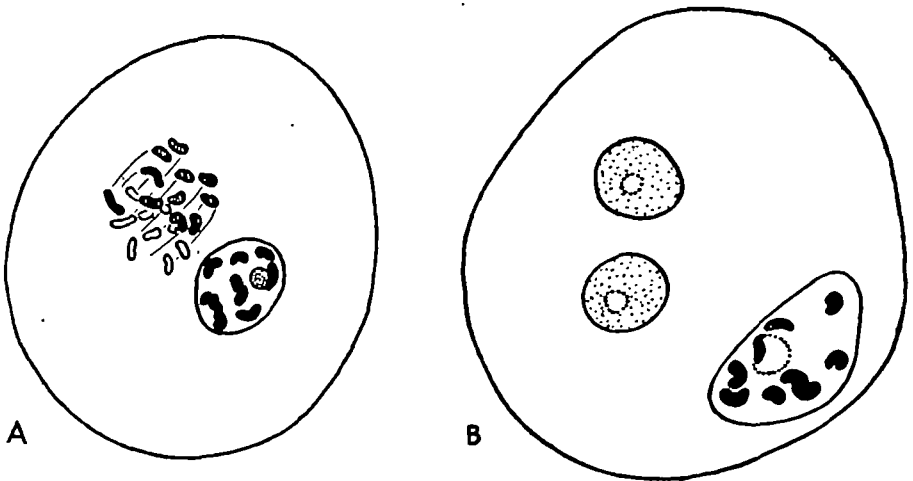


FIG. 10. Two stages of polymitosis in pollen of a diploid with undifferentiated nuclei. ($\times 2,200$.)

8. ISOCHROMOSOMES IN EQUILIBRIUM

Without undue hazard we may assume that the isochromosomes of *Nicandra* have arisen from ordinary chromosomes of the same permanent type as its autosomes. Such an origin of isochromosomes is now fairly well understood elsewhere (Darlington, 1940; Rhoades, 1940; Giles, 1943; Darlington and La Cour, 1944). Misdivision of the centromere, of an ordinary two-armed chromosome at meiosis is due either to failure of pairing (seen in *Tulipa*, *Pisum*, and here in *Nicandra* and to be inferred in *Datura* and *Zea Mays*) or to a failure of co-orientation of any chromosome (*Fritillaria*) or only of the longest (*Gasteria*). By misdivision telocentric chromosomes arise which at once or later undergo sister reunion of chromatids within the centromere and thus turn themselves into isochromosomes.

Isochromosomes have been found in exceptional stocks not only in these plants but also in *Sorghum* (Darlington and Thomas, 1941), *Campanula* (Darlington and La Cour, unpubl.), and, we may suppose, as the attached -X chromosomes of *Drosophila*. In none of these, however, does the isochromosome show the evidence of a long past or the promise of a long future. On the contrary where an established chromosome, the Y of *Drosophila melanogaster*, shows signs of having arisen from an isochromosome it also shows signs of the impermanence of its isogenetic character. It has lost most of one arm.

What then are we to assume of the isochromosomes in *Nicandra*? At meiosis they show signs of the abnormal centromere that we expect from an origin by misdivision. The end segments, between which crossing over takes place, must be identical. The correspondence in position of the two nucleolar organizers shows that they are included in these segments. The equal length of the two arms, however, goes farther: it suggests that their original identity has been preserved—preserved, that is, throughout the species and over a great space of time. Such an identity would not preclude the possibility of differentiation between the isochromosomes of different strains. This possibility might seem even more important since the isochromosomes are likely to undergo reconstruction following misdivision (Fig. 2). Yet there are no differences of behaviour between strains and there is therefore little likelihood of irregularity in crosses between strains.

Our problem is thus to find out why chromosomes which in themselves are peculiarly unstable, nevertheless, as part of the heredity of the species both in nature and in cultivation, are peculiarly stable. To do this we must discover what use the special properties of these chromosomes serve in the individual and in the species.

In the individual we see that they are uniformly necessary; that their optimum dose is the normal fourfold dose found in the diploid; but that a twofold dose gives viable results. This condition points to two analogies. The first is with the X-chromosomes in Orthoptera and elsewhere, and implies the action of Muller's principle (1932) of dosage compensation with the dosage

rates of two-and-four instead of one-and-two. The second analogy is with the supernumerary but profitable fragments of maize and Sorghum. These, however, can exist in any dosage proportion to the autosomes from nil to equality or beyond. They are non-specific and heterochromatic.

Taking both these analogies into consideration we must regard the isochromosomes of *Nicandra* as adapted to a special function. This function depends, however, on the advantage of the species rather than of the individual. It is that the errors of segregation of the isochromosomes lead to a heterogeneous progeny from homozygous individuals and in homogeneous strains. The mutants, however, have the faculty of reverting to type: 20 gives 19 and 19 gives 20 again. The heterogeneity affects particularly the rate of germination and thus enables a single homozygous plant to distribute its progeny over a greater number of years than it otherwise could. It can spread itself in time as well as in space. Such a property, analogous to that of 'hard seeds' in the Leguminosae, might well confer an advantage of decisive value to a species like *Nicandra physaloides* which is an isolated relic. The lower vitality and delayed germination of the deficient individuals will increase, in the right conditions, the chance of survival of the species while reducing that of a part of the individuals.

The condition of *Nicandra* is at present unique in our knowledge. We must not exclude the possibility, however, that isochromosome formation is a frequent process in nature even though it is rarely stabilized. Chromosomes with defective centromeres (such as the long chromosomes of *Gasteria*) may often have been derived from isochromosomes by loss, as the Y in *Drosophila* appears to have been. Species may use isochromosome formation as a recurring means of genetic reconstruction. Such a reconstruction must be intermediate in its effect on balance between the reduplication of minor segments and secondary polyploidy. Like them it provides a universally available means of achieving a necessary adaptation to a sudden change of the environment.

SUMMARY

1. *Nicandra physaloides* in all its varieties normally has 9 pairs of autosomes and 1 pair of isochromosomes ($2n = 20$).
2. At meiosis the isochromosomes pair either inside (to give univalents) or outside (to give bivalents) or both. When univalents are lost, pollen and eggs are formed lacking an isochromosome altogether.
3. The deficient pollen dies, but the deficient eggs must be fertilized since seedlings of delayed germination include a proportion lacking one isochromosome ($2n = 19$).
4. Slight polymitosis of the pollen occurred in one diploid and binucleate pollen in another.
5. Tetraploids with 4 isochromosomes ($2n = 40$) show the expected configurations and chiasma frequencies. The isochromosomes, as in diploids,

show an advantage of pairing inside over outside. The attachment of the arms thus facilitates inside pairing.

6. A triploid had asynapsis of the autosomes with normal chiasma formation in the isochromosomes. This is attributed to an accentuation in the advantage of attached arms as partners where there is a time limit to pairing.

7. The value of isochromosomes to *Nicandra* as of B chromosomes to maize is in securing heterogeneity. But in *Nicandra* this is a means of survival of the species rather than of the individual for the delayed germination which reduces the survival value of the deficient individuals increases the survival value of the species producing them.

LITERATURE CITED

- BELLING, J., 1927: The Attachment of Chromosomes at the Reduction Division in Flowering Plants. *Journ. Genet.*, xviii. 177-205.
- DAHLGREN, O. K. V., 1924: Kreuzungskleinigkeiten. *Hereditas*, v. 222-30.
- DARLINGTON, C. D., 1940: The Origin of Isochromosomes. *Journ. Genet.*, xxxix. 351-61.
- and LA COUR, L. F., 1942: The Handling of Chromosomes. Allen & Unwin, London.
- , 1945: X-ray Breakage and the Nucleic Acid Metabolism. *Journ. Genet.*, xli. 180-267.
- and THOMAS, P. T., 1941: Morbid Mitosis and the Activity of Inert Chromosomes in Sorghum. *Proc. Roy. Soc. B*, cxxx. 127-50.
- and UPCOTT, M. B., 1941: The Activity of Inert Chromosomes in *Zea Mays*. *Journ. Genet.*, xli. 275-96.
- FRANKEL, O. H., DARLINGTON, C. D., and LA COUR, L. F., 1940: The Causal Sequence of Meiosis I. *Journ. Genet.*, xli. 9-64.
- GILES, N., 1943: The Origin of Isochromosomes at Meiosis (*Gasteria*). *Genetics*, xxviii. 512-24.
- JANAKI-AMMAL, E. K., 1932: Chromosome Studies in *Nicandra physaloides*. *Cellule*, xli. 89-110.
- , 1941: The Breakdown of Meiosis in a Male-sterile *Saccharum*. *Ann. Bot., N.S.*, v. 83-8.
- LEMOINE, H., 1906: *Nicandra violacea*. *Rev. Horticole*, 1906, 208-9.
- LEVAN, A., 1940: The Cytology of *Allium amplexans*, &c. *Her.*, xxvi. 353-94.
- LEWIS, D., 1944: The Incompatibility Sieve for Producing Polyploids. *Journ. Genet.*, xlv. 261-4.
- MULLER, H. J., 1932: Further Studies on the Nature and Causes of Gene Mutations. *Proc. 6th Int. Cong. Gen.* Ithaca, 213-55.
- RAMANUJAM, S., and PAL, B. P., 1940: Asynapsis in Chilli (*Capicum annuum*). *Curr. Sci.*, iii. 126-7.
- RHOADES, M. M., 1940: Studies of a Telocentric Chromosome in Maize, &c. *Genetics*, xxv. 483-520.

