

# CYTOGENETICAL STUDIES IN *DATURA*

## I. Cytology of the Parents and of the F<sub>1</sub> Hybrid between *Datura fastuosa* and *Datura* sp.

BY PROF. T. S. RAGHAVAN, M.A., PH.D. (LOND.), F.L.S.

AND

A. R. SRINIVASAN, M.Sc.

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### I. Introduction

PLANTS belonging to the Solanaceæ have been, of all the families, the most widely investigated by the cytogeneticists. Of the different genera, the genus *Datura* would appear to have attracted their attention only comparatively recently. From the available literature, the earliest cytogenetical work on *Datura* seems to be Blakeslee's (1922), where he describes the variations in *Datura* as correlated to changes in chromosome numbers. From that year onwards, a number of papers by Blakeslee and his co-workers on many species of *Datura* have been published. The field that they have covered is wide, but mainly it can be said that they have studied mutants of *Datura*, either naturally occurring or artificially induced. Haploids of *Datura*, chiefly of hybrid origin, have been studied by Satina *et al.* (1937). Blakeslee and his co-workers (1936) published an account of investigation on *Datura*, where they have described the various methods by which the study of chromosomal differences between species of *Datura* can be made. Stuart Gager

and Blakeslee (1927) have described the nature of mutations in the chromosomes and among the genes, caused through exposure to Radium rays. Colchicine treatment and subsequent formation of chimeras with induced polyploid numbers, have been studied in *Datura stramonium* by Satina *et al.* (1941). Chromosome deficiencies due to the above cause are dealt with in a subsequent paper by Bergner *et al.* (1941).

The materials for the present investigation are two of the locally available species of *Datura*. The character contrasts in these two plants were so marked, that these formed good materials for a study of the inheritance of these characters. A detailed description of the parental species and the  $F_1$  hybrid is given below. The cytology of the parents and of the hybrid is also described.

## II. Description of the parents and the hybrid

### (a) *Datura fastuosa* (the black form), Pl. II, A and B; 2

A large shrub with crookedly-branching very dark purple stems, growing to 5-6 feet in height. Leaves large, ovate, sinuate. Flowers are solitary, large, purple and nearly  $8\frac{1}{2}$  inches long, on short pedicels. Calyx, tubular with five triangular lobes. The base of the calyx is persistent. Corolla consists of five petals, gamopetalous, trumpet-shaped, purplish outside and whitish inside, with plicate aestivation in the bud stage. Stamens are also five, filaments slightly shorter than the corolla, with long longitudinally dehiscing anthers. Ovary superior, bicarpellary, syncarpous, with fleshy branched placenta and numerous ovules. The wall of the ovary is thrown into numerous closely-set papillæ, which become straight, sharp and stout prickles, when the ovary ripens into a fruit. The fruit is a loculicidally septifragal capsule, big and ovally globose.

### (b) *Datura sp.* (the white form), Pl. II, A and B; 1

This parent is a short shrub growing to a height of 2-2½ feet. It is spreading in habit. The stems are green in colour. The flowers are smaller than those of the other parent, of length varying from 6-6½ inches. The colour of the corolla is yellowish-white. In the fruits, the prickles are thinner and shorter and less closely set than in the case of the other parents. The fruits are globose.

### (c) *The F<sub>1</sub> hybrid*, Pl. II, A and B; 3

This shows mostly characters intermediate between those of the parents. The plant grows erect and at the basal portion, the branches show a spreading nature. The stem is, however, dark-purple in colour and the average

height of the plant is about 4-4½ feet. The flowers are purple in colour and in length they are shorter than the black parent but longer than those of the white parent. The fruits show intermediate characters between those of the two parents.

There is nothing worth any special mention about the technique of crossing. The technique previously described for *Nicotiana* (Raghavan and Srinivasan, A. R., 1941 *b*) was followed.

### III. Cytological technique

Root-tips of the parents and the hybrid were obtained from plants grown in pots in the Botanical Gardens, Annamalainagar. These were fixed in Müntzing's modification of Navashin's fluid. Stages for meiotic studies were determined through acetocarmine examination and anther-smears were fixed in Belling's Navashin fluid, or fixed in Müntzing's fluid. The fixed materials were embedded in paraffin following the usual schedule and sections were taken at thicknesses varying from 10-18 microns. Both the smears and the sections were stained in Haidenhein's iron-alum hæmatoxylin.

### IV. Genetical

The  $F_1$  hybrid is intermediate between the two parents as regards most of the characters. However, a few features of the black parent are found to be dominant over those of the white in the  $F_1$  generation. The following table shows the important features of contrasts between the parents and their expression in the  $F_1$  generation.

TABLE I

Nature of the characters	Black parent	White parent	$F_1$ hybrid
Colour of the stem ..	Dark purple	Green	Dark purple
Habit .. ..	Tall, 7' and erect	Short, 3' in height and spreading	Tall and spreading, 4-5' in height
Length of the flowers ..	Long, 8½"	Short, 6"	8"
Colour of the corolla ..	Purplish outside and whitish inside	Yellowish-white	Purplish outside and whitish inside
Nature of fruits .. ..	Ovally globose, 2½" long	Round, 1½" long	2" long.

Thus features of colour (of stem and of flowers) appear to be an expression of the dominance of the black parent over the white. Other features of the hybrid are intermediate.

*V. Cytology of the parents**(a) Cytology of Datura fastuosa (black)*

*Somatic chromosomes.*—Fig. 1 represents the somatic metaphase plate of this parent. The twenty-four chromosomes can be grouped under four types, which are as follows:—

*Type A.*—Longest chromosomes, 4.5 microns in length and with median constrictions.

*Type B.*—Slightly shorter chromosomes, 3.7 microns long and with sub-median constrictions.

*Type C.*—Still shorter ones, 3 microns long and possessing sub-terminal constrictions.

*Type D.*—Shortest chromosomes, with a length of 2.3 microns having sub-terminal constrictions.

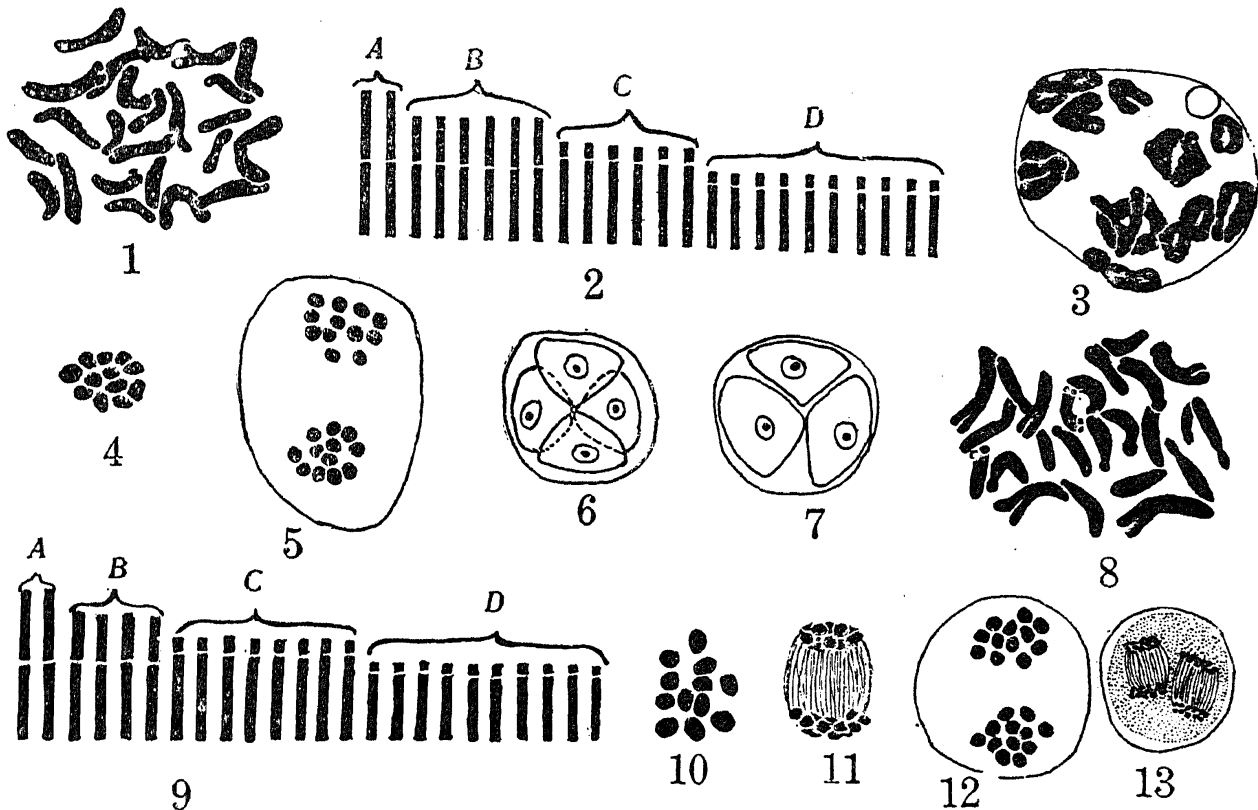
Out of the twenty-four chromosomes, two are of the A-type, six of the B-type, six of the C-type and the remaining ten of the D-type. Other features such as secondary constrictions, satellites, etc., could not be seen. Fig. 2 shows the idiogram representation of the complement.

*Meiosis—Diakinesis* (Fig. 3).—During diakinesis, the twenty-four chromosomes form twelve bivalents. A thorough examination of more than fifty diakinesis figures revealed that associations of more than two chromosomes were absent. Hence multivalent formation can be said to be totally non-existent. Of the twelve bivalents, one is large, possessing four chiasmata, two terminal and two interstitial. This probably arises from the two longest A-chromosomes which have been found to have median constrictions. Two other bivalents have three chiasmata, one interstitial and two terminal. Four ordinary ring-bivalents were met with, each having two terminal chiasmata. The remaining five bivalents form a single terminal or subterminal chiasma each. The total number of chiasmata in the black parent is twenty-three, of which four are interstitial. Thus conjugation is complete and the formation of bivalents with more than two chiasmata presumably shows the close relationship of the parental chromosomes.

*First division.*—The nucleolus and the nuclear membrane disappear and the nucleus after a short prometaphase, enters upon the first metaphase stage (Fig. 4). Twelve bivalents are arranged in the equatorial plate. No associations were found between the bivalents. Disjunction is normal.

*Second division.*—A short interphase intervenes between the two divisions, after which the chromosomes which disjoined in the first division

undergo homotypic division. In the second metaphase stage (Fig. 5) twelve chromosomes were seen at each of the two poles of the pollen mother cell. The second division is normal and tetrads of both the iso-bilateral and the tetrahedral types are produced (Figs. 6 and 7).



FIGS. 1-13

FIGS. 1-7. *Datura* (black)

Fig. 1. Somatic plate.  $\times 4400$ . Fig. 2. Idiogram representation of the chromosome complement. Fig. 3. Diakinesis.  $\times 4400$ . Fig. 4. M I plate.  $\times 2200$ . Fig. 5. Second metaphase.  $\times 2200$ . Figs. 6 and 7. Tetrads.  $\times 1500$ .

FIGS. 8-13. *Datura* (white)

Fig. 8. Diploid complement.  $\times 4400$ . Fig. 9. Idiogram representation of the same. Fig. 10. First metaphase.  $\times 2200$ . Fig. 11. Anaphase I.  $\times 1500$ . Fig. 12. Second metaphase.  $\times 2200$ . Fig. 13. Anaphase II.  $\times 1500$ .

### (b) Cytology of *Datura* sp. (white)

*Somatic chromosomes.*—The diploid complement of this parent is also made up of twenty-four chromosomes (Figs. 8 and 9). There are two chromosomes of the A-type, four of the B-type, eight of the C-type and ten of the D-type. There would appear to be difference only in the number of the two middle types of chromosomes (*i.e.*, B- and C-types of 3.7 and 3 microns length respectively). Whereas in the black parent there are six of each of these types of chromosomes, in the white parent, there are four of the B-type and eight of the C-type.

*Meiosis*.—Twelve bivalents are formed by the twenty-four chromosomes as shown in Fig. 10, which represents the polar view of the first metaphase plate. So conjugation in this species also is complete. Anaphasic separation (Fig. 11) is regular in the first division and the disjoined chromosomes organize two nuclei during interphase. During second metaphase (Fig. 12) twelve chromosomes are seen at each of the two poles. Second anaphase is also regular (Fig. 13), and normal tetrads are formed.

#### VI. Cytology of the hybrid

*Somatic chromosomes*.—The somatic chromosomes of the hybrid are represented in Figs. 14 and 15. The four types into which the parental chromosomal complements were analysed, could be recognized here also. In the somatic complement of the hybrid, there appear the two longest chromosomes, *i.e.*, of the A-type. Similarly there were ten short chromosomes—*i.e.*, of the D-type—about 2·3 microns long, so that in these two types there is complete identity between the parents and the hybrid. But in the nature of the two intermediate types there is some difference, for in the hybrid, we usually find only five chromosomes of the B-type (3·7 microns long) and seven chromosomes of the C-type (3 microns long). The different types of chromosomes and their number as occurring in the two parents and the hybrid, are shown in the following tabular statement.

TABLE II

Chromosome length and nature of constriction	White parent $2n = 24$	Black parent $2n = 24$	F <sub>1</sub> hybrid $2n = 24$
<i>A-type</i> .—4·5 microns and median constrictions .. .. .	2	2	2
<i>B-type</i> .—3·7 microns and sub-median constrictions .. .. .	4	6	5
<i>C-type</i> .—3 microns and sub-terminal constrictions .. .. .	8	6	7
<i>D-type</i> .—2·3 microns and sub-terminal constrictions .. .. .	10	10	10

The occurrence of five B-type and seven C-type chromosomes in the hybrid is as it should be, if we made a comparative study of the somatic complements of the parents that have entered into the formation of the hybrid complement. The haploid complement of the white parent should consist of the following chromosomes: one chromosome of the A-type, two of B-type, four of C-type and five of D-type. Similarly the black

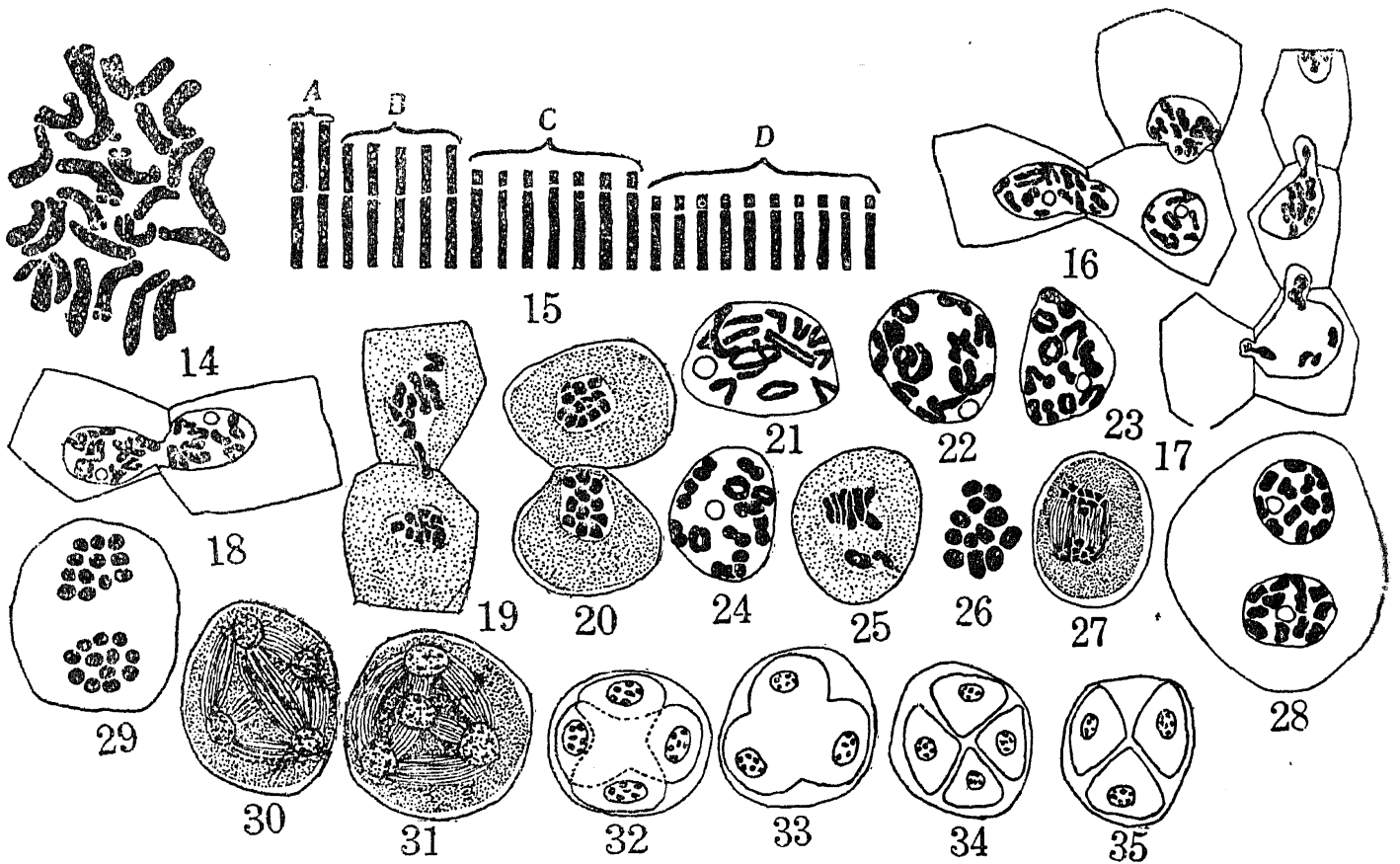
parent in its gametes should contain a genome composed of one chromosome of A-type, three of B-type, three of C-type and five of D-type. As these two gametic chromosome sets together compose the diploid complement of the hybrid, the hybrid complement comes to contain two chromosomes of the A-type, five of B-, seven of C- and ten of D-types.

Since the two gametic complements are identical to one another except in the number of the B- and C-types, we naturally find that in the hybrid these two types of chromosomes alone show a slight difference in their constitution. Generally, the prevalence of these odd numbers of chromosomes amongst these types may be expected to cause some degree of asynapsis resulting in the formation of univalents. But the difference between the B- and C-types is so slight that they may be expected to conjugate with one another without difficulty. That is why, we find meiosis regular in the main. The stray occurrence of trivalents which brings in its trail naturally the formation of univalents also, justifies this presumption.

*Meiosis*.—Stages earlier than diakinesis were not studied. During diakinesis, the phenomenon of cytomixis was found to occur frequently. It is interesting to note in this connection, that cytomixis was completely absent in the parents while it is so common in the hybrid. This only goes to support the view expressed in a previous paper (Raghavan and Srinivasan, 1941 *a*), that this phenomenon is associated with hybridity. Nevertheless apparently pure species have been found to exhibit this phenomenon as in the case of *Tridax* (Raghavan and Venkatasubban, 1941). Less frequent occurrence of cytomixis has been recently reported in the species, *Guetarda speciosa* (Raghavan and Srinivasan, 1941 *b*) and *Portulaca tuberosa* (Raghavan and Srinivasan, 1941 *c*). Nandi (1937) has recorded cytomixis during diakinesis stages in *Oryza* and is of opinion that it leads to polyploid gamete production through the formation of bi-nucleate pollen mother cells. X-rayed materials of pure species show the phenomenon of cytomixis to an extreme degree, e.g., *Capsicum* (Raghavan and Venkatasubban, 1940).

Cytomixis in the present case is very interesting. In Fig. 16, nuclear extrusion takes place into a single cell from two adjacent cells. In Fig. 17, nuclear matter from a single cell is extruded at the same time into two cells on either side of it. This recipient cell is found to transfer its chromatic material to the next cell and so on. Normal cytomixis between two adjacent cells has also been met with (Fig. 18). In the prometaphase stages also this phenomenon was found to occur (Fig. 19). Fig. 20 shows pollen mother cells at first metaphase stage, one of the metaphase plates showing a tendency to migrate. The relegation of two of the bivalents to a peripheral position

while the rest occupy the equatorial plate (Fig. 25), may also be regarded as showing an abortive attempt at cytomixis. In the present case the extent to which cytomixis occurs decreases as the pollen mother cells advance into the later stages.



FIGS. 14-35. Cytological stages of the  $F_1$  hybrid

Fig. 14. Somatic plate.  $\times 4400$ . Fig. 15. Idiogram representation of the same. Figs. 16-20. Stages of cytomixis.  $\times 1500$ . Figs. 21-24. Diakinesis stages. In Fig. 23 a trivalent and a univalent are shown. All the figures to the magnification  $\times 2200$ . Figs. 25 and 26. Metaphase I.  $\times 2200$ . Fig. 27. Anaphase I.  $\times 1500$ . Fig. 28. Interphase.  $\times 2200$ . Fig. 29. Second metaphase.  $\times 2200$ . Figs. 30 and 31. Second telophase.  $\times 1500$ . Figs. 32-35. Furrowing and tetrad formation.  $\times 1500$ .

Normal diakinesis was also quite common. Fig. 21 shows a P.M.C. at early diakinesis in which the synapsing chromosomes are long and slender. Of the twelve bivalents, three are of the ring-type, each with two terminal chiasmata. All the other bivalents have one terminal chiasma each. At a later stage, the chromosomes shorten and thicken (Fig. 22). In this case one of the bivalents was found to have an interstitial chiasma. Three ring bivalents were met with here also. In one case, a trivalent and a univalent were found along with ten bivalents, but such cases were very rare (Fig. 23). At later diakinesis, the configuration of the bivalents becomes clear (Fig. 24), when three ring bivalents and nine rod bivalents



are observed. Thus during diakinesis stages the meiosis shows normal conjugation between the chromosome sets of the parents. The total number of chiasmata is fifteen, interstitial chiasmata being almost absent.

At first metaphase all the twelve bivalents are arranged in the equatorial plate (Fig. 26). Very occasionally however, one or two of the bivalents are found to be located away from the equatorial plate (Fig. 25). First anaphase is regular but for the somewhat late disjunction of one of the bivalents (Fig. 27).

Interphase follows the first division and the nucleus assumes a globular appearance (Fig. 28). The constricted appearance of the chromosomes is characteristic of the interphase nuclei. The chromosomes are peripherally disposed at equal intervals from each other. The second prometaphase follows, but is of very short duration. Soon the nuclear membrane disappears and these constricted bodies contract and enter upon the second metaphase. Twelve chromosomes at each end could be recognized as seen from the pole (Fig. 29). During second telophase, the chromosome groups are arranged either in the iso-bilateral or in the tetrahedral pattern (Figs. 30 and 31 respectively).

Quadripartition of the tetrads is through furrowing (Figs. 32 and 33). The method of formation of iso-bilateral tetrads conforms to that described for *Nicotiana glutinosa* (Raghavan and Srinivasan, 1941 *a*). Normal tetrads of both iso-bilateral and tetrahedral types are formed.

### VII. Discussion

Interspecific hybrids between parents with the same number of chromosomes are not rare. These are interesting in the variety of genetical and cytological features that they present to us, such as, fertility or otherwise of the  $F_1$  hybrids, chromosome conjugation, abnormalities of meiosis, etc. There is a gradation from fully fertile hybrids to completely sterile ones. This only shows that the mere identity in the number of chromosomes is not enough by itself for the complete pairing of chromosomes. The degree of fertility is, in almost all cases, determined by the degree of conjugation between the parental chromosomes. In completely sterile hybrids, either conjugation is absolutely lacking, or weak conjugation takes place to such a low extent, as can be considered to be no better than asynapsis. Hybrids of this latter-mentioned type are many. Ramanujam (1937) obtained a hybrid between *Oryza sativa* ( $n=12$ )  $\times$  *O. officinalis* ( $n=12$ ), in which during diakinesis twenty-four unpaired chromosomes were met with. In some cases loosely formed bivalents were found and that too only to a low degree. This hybrid was found to be sterile. Crosses in *Nicotiana*, such

as those between *N. sylvestris* ( $n=12$ )  $\times$  *N. glutinosa* ( $n=12$ ); *N. rustica* ( $n=24$ )  $\times$  *N. tabacum* ( $n=24$ ) and *N. Raimondii* ( $n=12$ )  $\times$  *N. glauca* ( $n=12$ ) have led to similar results (Goodspeed, 1934).

Partial pairing between chromosomal sets of the parent species has been observed in other cases. Hybrids between species of *Lactuca* (Whitaker and Thompson, 1941), *L. tatarica* ( $n=9$ )  $\times$  *L. indica* ( $n=9$ ) showed  $7_{11} + 4_1$ , while  $F_1$  of *L. sativa*  $\times$  *L. vivosa* (both  $n=9$ ) showed  $8_{11} + 2_1$ . In spite of such partial pairing, the hybrids were found to be sterile. In hybrids of *Nicotiana* (Goodspeed, 1934), *Crepis* (Babcock and Emsweller, 1936), *Aegilops* (Percival, 1930) and *Gossypium* (Webber, 1935), such partial pairing between parental chromosomes resulting in varying numbers of bivalents and univalents were met with.

In the present case however, the parental chromosome sets seem to be completely homologous to each other. This is proved by the fact that twelve bivalents are regularly formed. But the number of chiasmata in the diakinesis stages of the hybrid is far less than in the parents. This probably signifies that, after all, two different parental sets of chromosomes—however homologous they may be—cannot pair as fully as those of the same parental species. In the present case, the average total number of chiasmata (during the mid-diakinesis stages) in the parental species is about twenty-three, of which four are of the interstitial type, the rest being terminal. In the hybrid, on the other hand, the number of chiasmata at about the same stage of meiosis, is about fifteen, all of which are terminal. Only very rarely do we see an interstitial chiasma. There is thus a marked decrease in the number of chiasmata in the hybrid, as compared to that of the parents. Also, interstitial chiasmata are conspicuous by their absence in the hybrid. Similar observations would appear to have been made by Goodspeed (1934) in interspecific hybrids of *Nicotiana* and his interpretation on these phenomena seem to indicate a correlation between these and the degree of chromosomal identity in the parental species. He says that "with increase in the number of bivalents (*i.e.*, in the homology of the parental chromosome sets), there is a disproportionately greater increase in the total number of chiasmata, because in certain of the bivalents two chiasmata are formed, and there is also an increase in the proportion of interstitial chiasmata. With almost complete pairing, the total number of chiasmata approaches that in the parent species...." Thus according to Goodspeed, the greater the number of chiasmata formed, the more homologous are the parental chromosomes. Viewed in this light, the chromosome sets of the two parents in the present case cannot be regarded as being so completely homologous.

For a casual observer the two parents used in the present cross, may appear to be only varieties of a distinct fundamental species. On closer scrutiny however, we find that the external features, such as the size and nature of the vegetative and the reproductive parts do not go to support this assumption. The large differences that exist between the two parents may, by themselves, be sufficient to classify them as distinct species. In addition to this morphological evidence, we have got cytological data also to prove the relative distance between the two parents. For example, a study of the somatic complements shows some differences in the chromosome morphology of the two parents. These differences have been pointed out already. Another, fact to reckon with, is that the number of chiasmata in the hybrid is much less than in the parents themselves; also interstitial chiasmata are characteristically absent in the hybrid. This, as has been pointed out already, suggests the somewhat distant relationship between the two parents. It will not therefore be wrong to regard them as two distinct species.

In spite of the above-mentioned differences, there is no deficiency in the homology between the parental chromosomes. Most probably the genes of the chromosomes are related to each other. Consequently, the meiosis is almost as normal as in the parent species and the  $F_1$  hybrid is fertile. Such fertile interspecific hybrids were met with in the case of *Nicotiana*, between *N. solanifolia* ( $n = 12$ )  $\times$  *N. paniculata* ( $n = 12$ ) (Goodspeed, 1934). Hybrids of *Lactuca*, other than those mentioned above, were found to be fertile, though to a lesser extent than the parents (Whitaker and Thompson, 1941).

Taxonomical relationships have recently been determined through studies in the chromosome behaviour of interspecific hybrids. Species whose chromosome sets pair freely with each other, are believed to be nearly related, the only difference between their chromosomes being of the nature of certain genetic factors. Partial or no pairing has been taken to represent distant relationship of the parents. In *Nicotiana* this principle has been found to be true. Thus, Goodspeed (1934) remarks "when two chromosome complements are capable of co-operating so that a normal soma is built, these chromosome sets have at least residual homology and presumably contain genes in common". He also says that "there is no justification for a disbelief in the significance of pairing as indicative of relationships in *Nicotiana*, because in other materials the evidence at present may not warrant such a conclusion". We find that this principle is applicable to the present hybrid in *Datura*. No abnormality of any serious nature having been observed in the hybrid, the two species may be looked upon as being very

nearly related to each other and can be traced back to identical origin. So, the above said principle that a close correlation exists between chromosome pairing and taxonomic relationship, is found to be true in the case of *Datura* also.

However, cases have been found where this principle presents obvious difficulties. Crosses by Clausen (1931) in *Viola* are examples of this. *Viola arvensis* ( $n = 17$ ) was crossed with *V. tricolor* ( $n = 13$ ) and the  $F_1$  hybrid showed  $13_{11} + 4_1$ , according to the Drosera type of chromosome conjugation. Thus the thirteen chromosomes of *V. tricolor* are homologous to thirteen chromosomes in the haploid set of *V. arvensis*. Another cross between *V. tricolor* ( $n = 13$ ) and *V. nana* ( $n = 24$ ) also showed perfect pairing between the *tricolor* and the *nana* chromosomes. If the hypothesis that chromosome pairing is indicative of the relationship between species be true, thirteen chromosomes of *V. arvensis* which paired with the chromosomes of *tricolor*, should conjugate with the thirteen chromosomes of *V. nana*, with which also the *tricolor* chromosomes exhibited pairing. This however was not the case and two to six bivalents were only formed in the hybrid between *V. nana* and *V. arvensis*. Even this bivalent formation might have been due to autosyndesis. In *Nicotiana digluta*, where the chromosomes are obviously homologous, non-pairing has been reported (Müntzing, 1935). Ramanujam (1937) is of opinion that chromosome conjugation may be influenced by genetic and environmental factors. According to him "while conjugation of chromosomes indicates a kind of homology between them, non-conjugation does not necessarily always mean non-homology".

The above instances show that much caution should be exercised while interpreting partial or no pairing with reference to relationship between parents of interspecific hybrids. But there seems to be no difficulty in interpreting complete pairing (as in the present case) as indicative of taxonomic nearness of the parental species.

### VIII. Summary

Two species of a local *Datura* were crossed and the hybrid compared with the parents.

The somatic chromosome complements of the parents were analysed and compared with that of the hybrid.

Meiosis in the parents and the hybrid is also described. Chromosomal pairing in the hybrid is discussed as throwing light upon the relationship between the parents.

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A. Photographs of whole plants of: 1. The white parent, 2. The black parent, and 3. The  $F_1$  hybrid



B. Photographs of flowers and fruits of: 1. The white parent, 2. The black parent; and 3. The  $F_1$  hybrid