SOMATIC INSTABILITY OF CHROMOSOME NUMBER IN *HYMENOCALLIS CALATHINUM*

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Received 1.vii.54

I. OBSERVATIONS

In the genus *Hymenocallis* the basic chromosome number appears to be 23 notwithstanding that in different species the chromosomes have quite different forms. They can be classified into two distinct groups by their chromosome complements. First, there is the complement where the chromosomes have mainly median or sub-median centromeres. Secondly, there is the complement where many of the chromosomes have terminal or nearly terminal centromeres. This complement, however, has been reported only in the tetraploid or near tetraploid forms. The chromosome numbers of these species are as follows* :—

Metacentric Group—

<i>H. amancaes</i>	$2n = 46$	†
<i>H. littoralis</i>	$2n = 46$	(Sato, 1938)
<i>H. lacera</i>	$2n = 69$	(Sato, 1938)
<i>H. senegambica</i>	$2n = 69$	†
<i>H. harrisiana</i>	$2n = 87$	† Fig. 1A
<i>H. speciosa</i>	$2n = \begin{cases} 46 \\ 90-100 \end{cases}$	$\begin{matrix} \text{(Sato, 1938)} \\ \text{(Heitz, 1926)} \end{matrix}$

Partly Telocentric Group—

<i>H. macrostephana</i>	$2n = 92$	Fig. 1B (Snoad, 1951)
<i>H. × Daphne (H. calathinum × H. speciosa)</i>	$2n = 92$	†

The second of these two types of chromosome complement has presumably evolved from the other by fragmentation. Subsequent hybridisation has introduced a basic number which happens to be a multiple of the original one but is composed of different chromosome types. This explains the absence of diploid plants with telocentrics.

* Root tips for squash preparations were fixed in La Cour's 2 BD and after one hour an equal quantity of 1 per cent. chromic acid was added to this fixative. Next day the roots were thoroughly washed in distilled water and treated in 1 part 20 vol. hydrogen peroxide and 3 parts saturated solution of ammonium oxalate for five minutes (Dr C. E. Ford, unpub.). The normal procedure for Feulgen squashes followed. 0.05 per cent. colchicine was used for the pre-treatment of root tips for some of the squash preparations.

Roots for embedding were fixed in La Cour's 2 BD, embedded, sectioned at 16-18 μ and stained with crystal violet. Pollen mother cells were treated as Feulgen squashes after fixation in acetic alcohol (1 : 3).

† New count.

There is a great range of basic numbers in the Amaryllidaceae and *Hymenocallis* would seem to be at the end of an evolutionary series rising from 6 to 23.

In addition to those species already mentioned, *Hymenocallis calathinum* is also found to contain many telocentric type chromosomes. An attempt to determine its chromosome number, however, can be very confusing. Four plants of one clone were examined. The cells in the root tips contain different numbers of chromosomes. The variation is from 23 to 83 chromosomes though the lower and consequently more unbalanced complements are far less frequent than the higher ones (graph 1). The examination of these complements does not reveal any pattern of chromosome distribution. They are

FIG. 1.—Root-tip mitoses.

(A) *Hymenocallis harrisiiana* ($2n = 87$). All metacentric chromosomes. $\times 1050$.

(B) *H. macrostephana* ($2n = 92$). Complement includes 27 telocentric chromosomes. $\times 1050$.

not apparently segregated into multiples of any basic number but distributed more at random. Similarly the abnormal cells are not confined to any one region of the root.

In colchicine-treated squash preparations of root tips many of the metaphase nuclei are obviously unusual. The shortened chromosomes are often segregated into two, or even three, distinct groups (plate, fig. 2) suggesting an irregular grouping of the chromosomes and the formation of separate nuclei at the previous telophase. Even taking the spindle inhibiting effect of the colchicine into account this peculiarity appears unusually evident. Accordingly, preparations of roots without pre-treatment—both sectioned and squashed—have been examined. Spindle abnormalities are then seen (plate, figs. 3 and 4). Some cells have two distinctly separate spindles and others

tripolar ones. In addition, many micronuclei of varying size are to be seen. The variability of the chromosome number is thus explained.

These split spindles allow the unhampered separation of the chromatids and their passage to the poles. At telophase, however, in many cells, presumably because of insufficient room, there is often

GRAPH 1.—Showing the frequency of different chromosome complements in *H. calathinum* at mitosis (94 cells counted) and meiosis (73 cells counted).

fusion of these irregularly distributed daughter groups. As a result two or more groups become enclosed within one cell wall and the higher chromosome numbers are restored. It is also highly likely that nuclei with low numbers are shortlived because of extreme unbalance.

This variation in chromosome number is also carried through the germ line so that the pollen mother cells contain differing numbers of chromosomes. The range of numbers at meiosis is smaller than

that at mitosis (graph 1). This provides further evidence of the instability of the smaller chromosome complements. The premeiotic cells have probably carried the variation through many cell generations but the conditions only permit the larger and more balanced numbers to propagate themselves.

The highest number of univalents seen in any pollen mother cell was four. This can perhaps be attributed to somatic pairing of the chromosomes so that spindle abnormalities lead to the segregation of mainly homologous pairs rather than to a random distribution. Somatic pairing has also been inferred in *Helianthemum* (Snoad, 1954) where, in some plants, meiotic pairing of reduced chromosome complements was higher than would be expected on random splitting of the spindle.

2. DISCUSSION

That the number of chromosomes within individuals—both plants and animals—is not wholly constant has been quite widely reported. These reports, however, deal mainly with increases in chromosome number to give multiples of the basic number. Thus, endomitosis has been observed in the tapetal cells of tomato (Brown, 1949) and *Spinacia* (Witkus, 1945) and also in the nurse cells of the ovary of *Drosophila melanogaster* (Painter and Reindorp, 1939). Polyploid and mixoploid tissues have been observed in the nymphs of *Romalea microptera* (Mickey, 1946). Polyploid cells have also been reported in mammals—in the liver (Bieseke, 1944) and in the blood (La Cour, 1944).

The frequency of reduced chromosome numbers within individuals is not so great. In adult human uterine epithelium in its proliferative stage and various embryonic tissues—skin, brain, liver, connective tissue, intestine, etc.—great sub-diploid variation has been found (Therman and Timonen, 1951). This variation was denied by Sachs (1954). He examined the normal endometrium in *Homo sapiens*, *Rattus norvegicus* and *Microtus agrestis* but no sub-diploid variation was apparent. The presence of polyploid cells was, however, confirmed.

A very wide range of chromosome numbers was found in colchicine-induced tetraploid *Ribes nigrum* bushes and in their progeny raised from seed (Vaarama, 1949). The chromosome numbers in the root tips varied from 4 to 32. The cause of this variation was attributed to the presence of two separate spindles in the cells—a similar condition to that in *Hymenocallis*.

Another report of chromosome mosaicism, this time of pollen mother cells, is that of Sachs (1952). It was found in 19 out of 24 amphiploids in *Triticum*, *Aegilops* and *Agropyron*. The reduced chromosome numbers ranged from 9 to 48 in the 8x plants and 10 to 40 in the 6x ones. As there were no mosaics in the root tips of these plants the presumed spindle abnormalities must have occurred late in development, i.e. during the premeiotic mitoses.

Plate

Mitosis in the root tips of *Hymenocallis calathinum*. Feulgen squash method.

- FIG. 1.—Colchicine-treated metaphase showing a highly reduced chromosome number of 34. $\times 1040$.
- FIG. 2.—Metaphase after colchicine treatment showing the segregation of the chromosomes into two distinct groups. $\times 1400$.
- FIG. 3.—Upper cell with a tripolar spindle, lower one with three separate spindles. $\times 1040$.
- FIG. 4.—Two separate spindles within one cell. $\times 1040$.

Sub-diploid variation of chromosome numbers has also been found in the pollen mother cells of *Helianthemum* (Snoad, 1954). Here also the spindle disturbances are assumed to have occurred during several premeiotic mitoses but they are also associated with a failure of wall formation so that plasmodial pollen mother cells are formed.

The nuclear behaviour in *H. calathinum* follows the pattern laid down by many of these examples. A disorganisation of the spindle's function brings about a variation in chromosome number but in *Hymenocallis* this variation appears to be more widespread than in any other organism so far investigated. As in the only other root-tip variation (*Ribes*) the high chromosome number may not only be responsible for the origin of the spindle abnormalities but also for the survival of so many nuclei with reduced chromosome numbers.

3. SUMMARY

1. No constant chromosome number is to be found in the root tips of *Hymenocallis calathinum*. The numbers found range from 23 to 83.
2. A variation over a smaller range—from 69 to 86 chromosomes—is also found in the pollen mother cells.
3. Spindle abnormalities are seen to give rise to this variation in chromosome number.
4. There are different levels of tolerance to unbalanced chromosome numbers in anthers and roots.

Acknowledgment.—I wish to thank Mr L. F. La Cour for his helpful criticism and advice.

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