

SUPERPOSABLE PICTURES OF CHROMOSOME STRUCTURE OF *ALLIUM CEPA* IN FEULGEN AND HAEMATOXYLIN SQUASHES

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INTRODUCTION

THE hæmatoxylin squash method¹⁻³ gave elegant staining of the chromosomes and non-fading permanent preparations. The hydrolysis in N HCl at 60° C. for 10-12 min. prior to staining was reminiscent of a similar step in Feulgen procedure.⁴ Cells stained with leuco-basic fuchsin gradually fade on keeping. If pictures obtained with hæmatoxylin are superposable on those of Feulgen it would be a permanent record of the observations.

While hæmatoxylin stains both acidic and basic structures, the Schiff's reagent colours only structures containing DNA. Sections of fixed material hydrolysed as for Feulgen but stained with hæmatoxylin show both the chromatin and nucleoli in slightly differing shades while a counterstain is necessary to reveal the nucleoli in Feulgen preparations. If it is the fixative which renders the nucleoli stainable with hæmatoxylin even after hydrolysis, then, avoidance of conventional fixatives should enable obtaining hæmatoxylin preparations where the nucleoli would remain unstained and the details of chromosome structure and configuration superposable on those stained by the Feulgen technique.

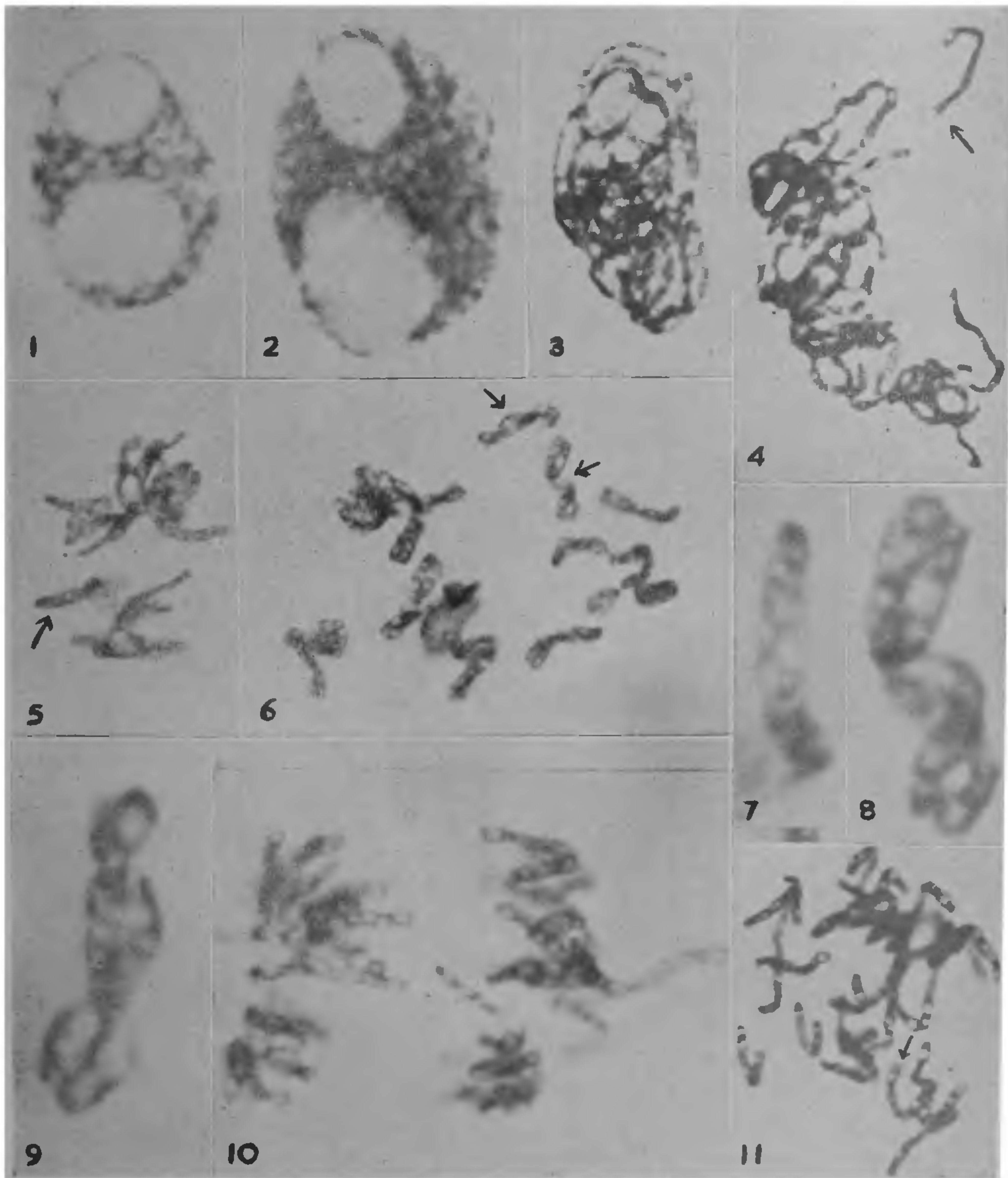
The most obvious procedure is to employ N HCl at 60° C. as the medium for fixation as well as hydrolysis.⁵ Fresh roots of *Allium cepa* exposed for 8 min. to hot HCl were washed in distilled water and stained either with leuco-basic fuchsin or with Heidenhain's hæmatoxylin. For Feulgen staining, the washed roots were transferred to leuco-basic fuchsin and kept in the dark for 3 hrs. After a wash in three changes of SO₂ water for a total duration of 30 min. they were rinsed in distilled water and squashed in 45% acetic acid under a coverslip. The slides and coverslips used should be coated with a thin layer of Mayer's albumen and then flamed. When the spreading of the cells appeared satisfactory, the coverslips were released in 40% alcohol. They were dehydrated in ascending grades of alcohol, then cleared in xylol and mounted in Canada balsam. Some of the material was counterstained with aqueous light-green. The procedure for making hæmatoxylin squashes was essentially the same as described previously.¹

Since there is a slight increase in the intensity of the stain when made permanent, the cells in the squash should be destained to a stage lighter than actually desired. This could be achieved by the control of the time of stay of the stained material in 45% acetic acid before squashing. The preparations could be made permanent by dehydration with either ethyl or tertiary butyl alcohol. When the latter is used, they could be mounted direct in Canada balsam dissolved in butyl alcohol. The use of ethyl alcohol necessitates clearing in xylol before mounting in Canada balsam.

OBSERVATIONS

The resting nucleus has a reticulated appearance in Feulgen (Fig. 1) as well as hæmatoxylin (Fig. 2) preparations and the areas occupied by the nucleoli appear unstained. The structural details of the chromosomes during the various phases of the division cycle are also superposable (Feulgen—Figs. 3, 5, 7 and 10; hæmatoxylin—Figs. 4, 6, 8, 9 and 11). During prophase, the chromosomes appear to consist of two chromatids and the free ends of one such pair are indicated by an arrow in Fig. 4. The general impression one gets is that the chromatids are relationally coiled.

The most unexpected discovery was that while the two chromatids of the metaphase chromosome showed relational coiling (Figs. 5, 8 and 9), each chromatid itself appeared linearly vacuolated reminiscent of descriptions of coiling of a pair of chromonemata.^{6,7} In other words, the appearances are suggestive of a quadri-partite structure of the metaphase chromosome.⁸ It need hardly be mentioned that hæmatoxylin pictures were superior to those of Feulgen in their clarity for demonstration of the relational coiling of the chromatids as well as the coiled pair of chromonemata. This is exemplified by enlargements of individual chromosomes (Figs. 7, 8 and 9) indicated by arrows in Figs. 5 and 6. At anaphase the daughter chromosomes show the same type of configuration as exhibited by the individual chromatids at metaphase. The free ends of the two chromonemata of an anaphase chromosome are indicated by an arrow in Fig. 11. The coiling of the chromonemata at meta- and anaphases appears to be of the caduceus type.⁹



FIGS. 1, 3, 5, 7 and 10. Direct N HCl-Feulgen Squash. Fig. 1. Resting nucleus, $\times ca. 2,500$. Fig. 3. Prophase showing the spiral structure, $\times ca. 1,500$. Fig. 5. Metaphase. One chromosome shows the quadri-partite structure, $\times ca. 1,500$. Fig. 7. Enlargement of the chromosome arm indicated in Fig. 5. $\times ca. 6,000$. Fig. 10. Anaphase. Most of the chromosomes consist of a pair of caduceusly coiled chromonemata, $\times ca. 2,900$.

FIGS. 2, 4, 6, 8, 9 and 11. Direct N HCl-Iron Haematoxylin Squash. Fig. 2. Resting nucleus, $\times 2,500$. Fig. 4. Prophase. Compare with Fig. 3. Arrow indicates the bifid end of a chromosome, $\times ca. 1,500$. Fig. 6. Metaphase. The chromatids show relational coiling. Each chromatid is composed of a pair of chromonemata, $\times ca. 1,500$. Figs. 8 and 9. Enlargements of two of the chromosomes indicated by arrows in Fig. 6. Note the relational coiling and quadri-partite structure, $\times ca. 6,000$. Fig. 11. Anaphase. The free ends of one pair of chromonemata are indicated by an arrow, $\times ca. 1,500$.

DISCUSSION

Squash techniques obviate the use of conventional fixatives so necessary to render a root suitable for serial sectioning. It is this advantage which enabled devising a procedure by which the details shown by hæmatoxylin would only be those that are seen after Feulgen. Unexpectedly, the new method revealed also the structural details of the chromosomes during the various phases of mitosis. This was not very surprising since one of the methods used for revealing the spiral structure is exposure to fumes of concentrated HCl.^{7,8,10,11} But exposure to acid fumes was only a pretreatment which was followed by preservation in various fixatives. Accurate control of the pretreatment is rather difficult. A clear evaluation of the action of fixatives after such pretreatment is not also easy. These limitations are minimised in the new technique devised. The temperature and time of hydrolysis in N HCl of fresh roots could be controlled and there is no subsequent fixation. The results obtained are, therefore, easily reproducible.

Though the first description of the chromosome spiral by Baranetzky in 1880 was from damaged living cells, a wide variety of agencies have been used to improve its clarity for purposes of analysis.¹¹ Naturally, the spiral thus demonstrated is a vital artefact. The figures presented show that the same details of chromosome structure are seen in hæmatoxylin

and Feulgen preparations. But then, the agency producing the clarity of structural details is not the stains but the use of N HCl at 60° C. as the medium for fixation and hydrolysis.

The chromosome matrix appears Feulgen negative, the chromonemata alone being Feulgen positive. The clarity of pictures in which the free ends of the chromonemata lie separated (Fig. 11) suggests that the pairs of chromonemata are enclosed within a pellicle.¹² Other methods of approach are now being explored to evaluate whether the configuration of the coiling of the chromonemata is similar after pretreatment with different agencies and whether clear proof would be available for the presence of a pellicle.

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INDIA'S POPULATION ACCORDING TO 1961 CENSUS

PRELIMINARY figures of the population of India according to the census of February-March 1961 were published recently. (The figures for Manipur, Nagaland, and North-East Frontier Agency are not included.) The total population is 436,424,429, out of which 224,957,948 are males and 211,466,481 are females. The increase in population over the figures for 1951 is 77,207,524. This means that during the decade 1951-61 the population has increased by 21.49%. This growth is 61% faster than the rate at which the population grew between 1941-51 which was 13.34%. Out of the 436 millions, the rural population is 358 millions or 82% and the urban population is 78 millions or 18%.

Indian Union has an area of 1,127,345 sq. miles. The density of population is 384 per square mile as against 316 in 1951. Regarding sex-ratio, the number of females per 1,000 males is 940, as against 946 in 1951.

The distribution of population amongst the

15 States of the Union, and the percentage increase during the decade 1951-61 are shown in the following table.

TABLE I

| States | Area (in 1000 sq. miles) | Population (in millions) | Percentage increase over 1951 figure |
|-------------------|-----------------------------------|-----------------------------|---|
| Andhra Pradesh | .. 106.0 | 35.98 | 15.63 |
| Assam | .. 47.1 | 11.86 | 34.30 |
| Bihar | .. 67.2 | 46.46 | 19.78 |
| Gujarat | .. 72.1 | 20.62 | 26.80 |
| Jammu and Kashmir | | 3.58 | 9.73 |
| Kerala | .. 15.0 | 16.88 | 24.55 |
| Madhya Pradesh | .. 171.2 | 32.39 | 24.25 |
| Madras | .. 50.1 | 33.65 | 11.73 |
| Maharashtra | .. 118.9 | 39.50 | 23.44 |
| Mysore | .. 74.1 | 23.55 | 21.36 |
| Orissa | .. 60.2 | 17.57 | 19.94 |
| Panjab | .. 47.1 | 20.30 | 25.80 |
| Rajasthan | .. 132.2 | 20.15 | 26.14 |
| Uttar Pradesh | .. 113.5 | 73.75 | 16.67 |
| West Bengal | .. 39.9 | 39.97 | 32.94 |