Analysis of Y Chromosome Heterochromatin in *Rumex thyrsiflorus*

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Abstract. The Y chromosome heterochromatin in *Rumex thyrsiflorus* has been analyzed. In natural populations the Y chromosome shows a higher morphological variability than the X chromosome. The total duration of replication of Y chromosomes is about 2 hrs longer than that of euchromatin. Autoradiography with tritiated thymidine showed that chromocentres formed by Y chromosomes in interphase nuclei retain their heterochromatic form during DNA replication. — Y chromosome heterochromatin in interphase nuclei is stained pink, while the rest of the nucleus stains green after fast green-eosin staining for histones. — During the premeiotic stage of PMC development Y chromosomes are no longer visible as compact bodies and become more fuzzy in appearance. A diffuse state of Y coincides with intense RNA synthesis. Therefore genetic activity of Y chromosomes or their parts during premeiotic stage of meiosporogenesis is postulated (Summary see p. 352).

Introduction

*Rumex thyrsiflorus* is a dioecious species with the chromosome constitution XX + 12A = 14 in female and XYY + 12A = 15 in male individuals. Both Y chromosomes are completely heterochromatic and late replicating. In interphase nuclei of male plants as a rule two big chromocentres are formed by the Y chromosomes. This gives the opportunity to study more closely the duration of replication of Y chromosomes and the persistence of heterochromatin during DNA replication.

Recent studies show (Dronamraju, 1965; Schultz, 1965; Brown, 1966) that the inertness of heterochromatin is not absolute. During some periods of the cell cycle (Grüneberg, 1967; Back, 1969, in press) and during some period of ontogenesis (Hess, 1967; Hess and Meyer, 1968; Henning, 1967, 1968) heterochromatin can lose its compact state and exhibit genetic activity. Observations were therefore made concerning the behaviour of Y chromosome heterochromatin in Rumex in various tissues and during premeiotic and meiotic stages of development.

Material and Methods

Smear preparations of root tips were made in the routine way and stained with orcein or Feulgen. For the study of meiosis alcoholic hydrochloric acid
carmine was used after Snow (1963). Paraffin preparations for study of meiosis using standard fixatives and standard methods of staining were also made.

For staining histones by the fast green-eosin method according to Bloch (1966) tissues were fixed in 10% formalin neutralized either with CaCO₃ or 1 N NaOH. The material was dehydrated, embedded in paraffin and sectioned. The thickness of sections was 15 μ.

The standard procedure for staining with fast green eosin was as follows (Bloch, 1966)
1. Deparaffinize sections.
2. Hydrolize 15 min in 5% trichloroacetic acid 90—100°C to remove nucleic acids.
3. Impregnate with celloidin by running quickly through higher alcohols to methyl benzoate, then 1% celloidin in methyl benzoate, 1/2 hour. Rinse in methyl benzoate, alcohols, and bring to 70% alcohol.
4. Rinse for three 10 min in 70% ethanol, 0—5°C to remove trichloroacetic acid.
5. One half hour in 0.1% fast green FCF, 0—5°C buffered at pH 8.2—8.3 with tris HCl buffer 0.07 M.
6. Two hours 0—5°C in a mixture containing 0.05% fast green FCF and 0.05% eosin Y in the above buffer.
7. Differentiate 10 min in buffer, 0—5°C.
8. Ten minutes in 70% ethanol, 0—5°C to remove non specific acid dye.
9. Ten minutes in 95% ethanol, 0—5°C.
10. Complete dehydration and mount.

The fast green FCF and eosin Y were obtained from Edward Gurr, Ltd, London.

For observations of RNA synthesis during meiosis the flower shoots up to 5 cm long of male plants of *R. thyrsiflorus* were incubated 48 hrs in a solution of radioactive ³H-uridine (3 μc/ml) in White’s basic medium supplemented with 0.3 M sucrose, fixed in chilled acetic alcohol (1:3), dehydrated, embedded in paraffin and sectioned (thickness 5 μ).

The sections were covered with NTB-2 liquid emulsion and exposed in 4°C temperature for 6—12 weeks. After exposure the sections were developed in a Kodak D 19 developer and stained with methyl green pyronin (Unna-Pappenheim) obtained from Edward Gurr Ltd, London.

The duration of the S period in mitotic cells was measured by means of pulse and chase label as follows. The root tips were incubated for 1 hr in ³H-thymidine solution (2 μc/ml). After incubation the root tips were washed in a solution of cold thymidine (80 μg/ml). Before fixation they were treated for 4 hrs with 0.05% colchicine solution. The fixation was carried out in Carnoy’s fixative (ethanol 3: glacial acetic acid 1: chloroform 1). Root samples were fixed at two hour intervals up to 26 hrs. They were transferred to 95% ethanol and stored in a refrigerator at 0°C. All root tips were stained with Feulgen reaction and autoradiographed with Kodak AR-10 fine grain stripping film.

**Results**

**Localization of Heterochromatic Segments in the Karyotype of *R. thyrsiflorus***

Heterochromatic segments in the karyotype of *R. thyrsiflorus* have been located by means of cytological observations of prophase nuclei and by the method of Tjio and Levan (1950). The results were confirmed by autoradiographic investigations (Zuk, in press). It was found that both Y chromosomes are heterochromatic and late replicating. The short