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Autor: Leemann, Ursula / Ruch, Fritz

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DNA and base content in the nuclei and the sex chromatin of *Rumex acetosa*

von *Ursula Leemann* und *Fritz Ruch*

Institut für Allgemeine Botanik, Eidgenössische Technische Hochschule, Zürich

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Summary

The heterchromatic Y chromosomes of *Rumex acetosa* contain AT rich DNA. They can be easily identified in mitotic as well as interphase nuclei (as sex chromatin bodies) by double-staining with CMA and DAPI. The AT content in the female (mostly euchromatic) nuclei is approximately 60%, in the Ys approximately 80%. The 2C DNA content was determined as 7 pg. *Rumex acetosa* produces a UV absorbing substance which interferes to some degree with DNA staining especially after ethanol acetic acid fixation.

Introduction

The chromosomes and the sex determination of *Rumex acetosa* (2n = 12 + 2x; 12 + X + 2Y) were described by Ono (1935) and by Löve (1942). The sex of the plant is determined by the X/autosome ratio; the two Ys seem to play a minor role. As shown by Váňa (1972 a ,b) they are largely heterochromatic, while the X chromosome as well as the autosomes show few heterochromatic bands. Cell populations practically identical in the euchromatin, but differing in a sizeable amount of heterochromatin (table 1) are interesting objects for cytochemical investigations of heterochromatin. Male and female plants of *R. acetosa* were thus studied with banding dyes (Schweizer 1981).The fluorochrome pair Chromomycin A3 (CMA) and DAPI, which has been used for cytofluorometric base content determination (Leemann and Ruch, 1982), was of special interest.

Material and methods

Young leaves and root tips treated with Colchicine (0,05%) of *R. acetosa* were fixed overnight either with ethanol-acetic acid 3:1 (EA) or formalin 4% (buffered to pH 7), rinsed and stored in ethanol 70%.

Squash preparations were made in 45% acetic acid after heating for 3 min to 60 °C. After removing the coverslips on dry ice the slides were rinsed in ethanol and air dried. *Suspensions of stained nuclei* were preparad by grinding pieces of fixed leaves in a mortar in a few drops of dye solution. Root tips of *Vicia faba* treated similary (either squashed or ground) served as Standard object.

CMA and DAPI: the slides were stained either with one or consecutively with both fluorochromes. For CMA (100 μmg/ml McIlvaine buffer pH 7.0 containing 10 mM $MgCl_2$) the staining time was 30 min; with DAPI (2 μg/ml McIlvaine buffer pH 7.0) the preparations were stained for 20 min, or after CMA, for 3 to 6 min. The preparations were rinsed in buffer and mounted in glycerol/buffer mixture 1 : 1.

Quinacrine mustard (QM): the slides were stained for 20 min in QM (50 μg/ml Soerensen buffer pH 6.8), rinsed and mounted in glycerol buffer 1 : 1.

Feulgen-reaction: the tissue was hydrolysed in HCl either 1N at 60 °C, or 5N at 22°C for various periods of time, and stained according to the usual Feulgen procedure. It was squashed in 45% acetic acetic acid at room temperature, dehydrated and mounted in Fluormount.

Giemsa: the staining was carried out after hydrolysis with $Ba(OH)_2$ according to Stack & Comings (1979).

Microphotography was carried out with a Zeiss photo microscope III and with a Zeiss WL fluoroscence with vertical illuminator IV FL and HBO 50 merculy lamp.

Fluorescence measurements were made with a Zeiss cytofluorometer (Ruch and Trapp, 1972); the excitation filtres were BP 365/11 (DAPI) and BP 436/8 (CMA, QM) (Leemann and Ruch 1978).

Absorption measurements were made with a Zeiss scanning microspectrophotometer.

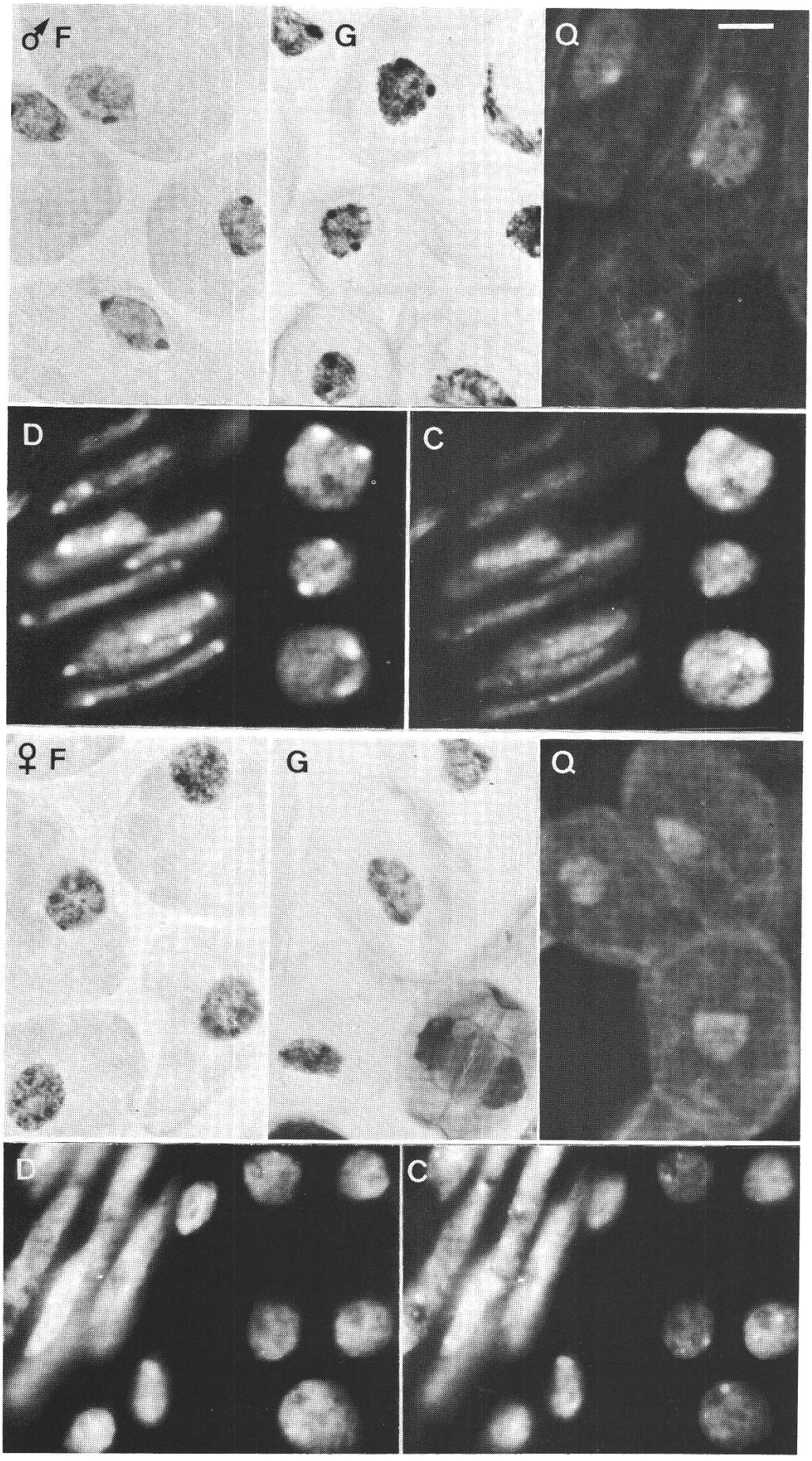
Results and Discussion

Morphology

Squash preparations from root tips as well as from leaves clearly show the difference between male and female (Fig. 1). The *interphases* of the male show two *sex chromatin bodies* that are of more or less spherical shape in the differentiated cells with staining procedures except CMA. In the root meristem these may appear rather fibrillar. In the nuclei of females they do not appear, but in both sexes a few smaller chromocenters can be discerned. Male and female plants are most easily distinguished with the *double staining DAPI/CMA*. The sex chromatin bodies fluoresce brightly with the AT dye DAPI, and weakly with the GC dye CMA. Two small chromocenters are also DAPI positive, while the two most prominent ones are CMA positive.

That the two large DAPI positive chromocenters are indeed the sex chromatin bodies is shown in *mitoses:* the two *Y chromosomes* fluoresce more strongly than the autosomes with DAPI, less strongly with CMA (Fig. 2). The X chromosomes stain with both dyes in the same way as the autosomes. Bands are not very clearly distinguishable, neither with the fluorochromes nor with Giemsa C-band staining. A karyotype is shown in Fig. 3, however, in some preparations the strong CMA band was localized on the long arm of one of the medium sized autosomes. The bands agree more or less, except for the centromeric regions, with the heterochromatin observed with aceto-orcein techniques by Váňa (1972b).

Fig. 1. Sex chromatin bodies in squash preparations of *Rumex acetosa* leaves fixed in ethanol acetic acid. Upper half: male, lower half: female plant. F: Feulgen; G: Giemsa; Q: Quinacrine mustard; D: DAPI; C: chromomycin; bar: 10 μm.


♂ F
G
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C
♀ F
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C

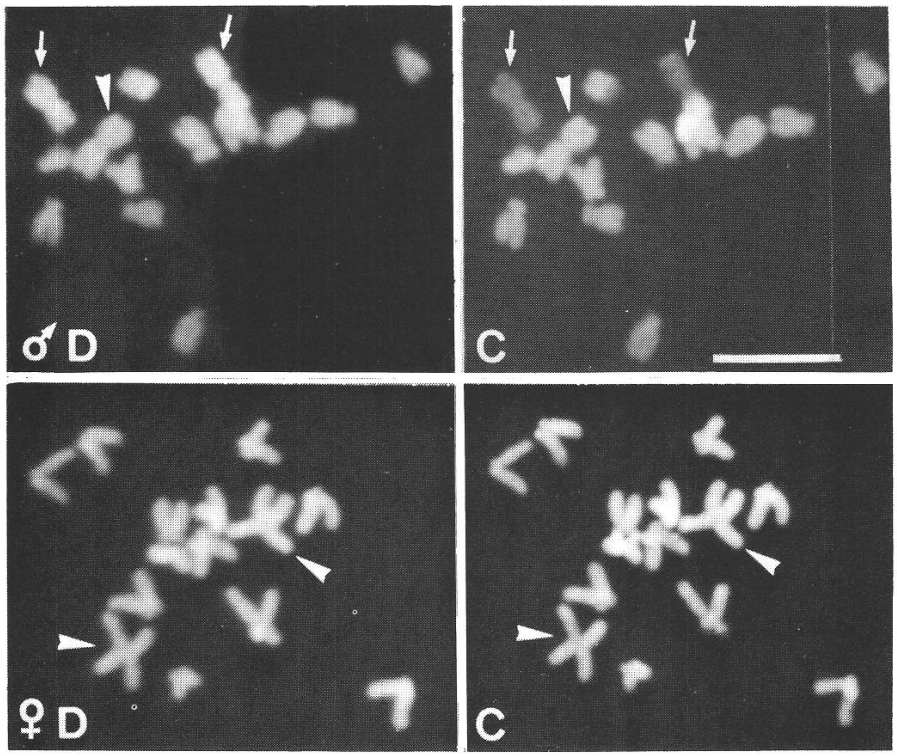

Fig. 2. Mitoses from *Rumex acetosa* root tip. D: DAPI; C: chromomycin. X chromosomes; Y chromosomes; bar: 10 μm.

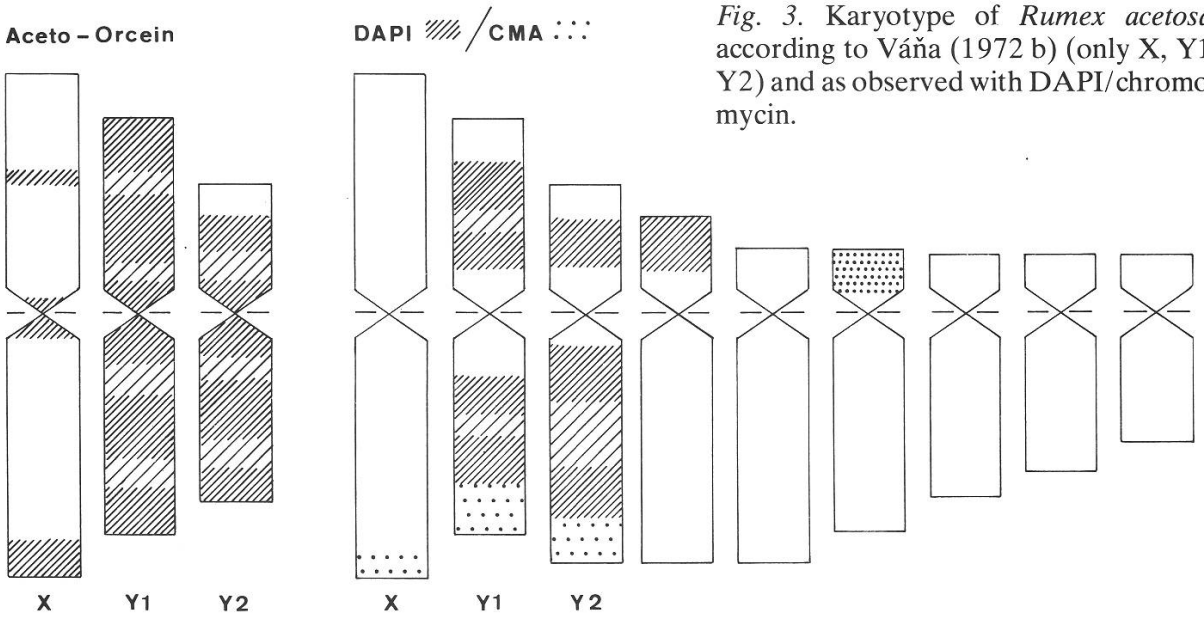

Fig. 3. Karyotype of *Rumex acetosa*, according to Váňa (1972 b) (only X, Y1, Y2) and as observed with DAPI/chromomycin.

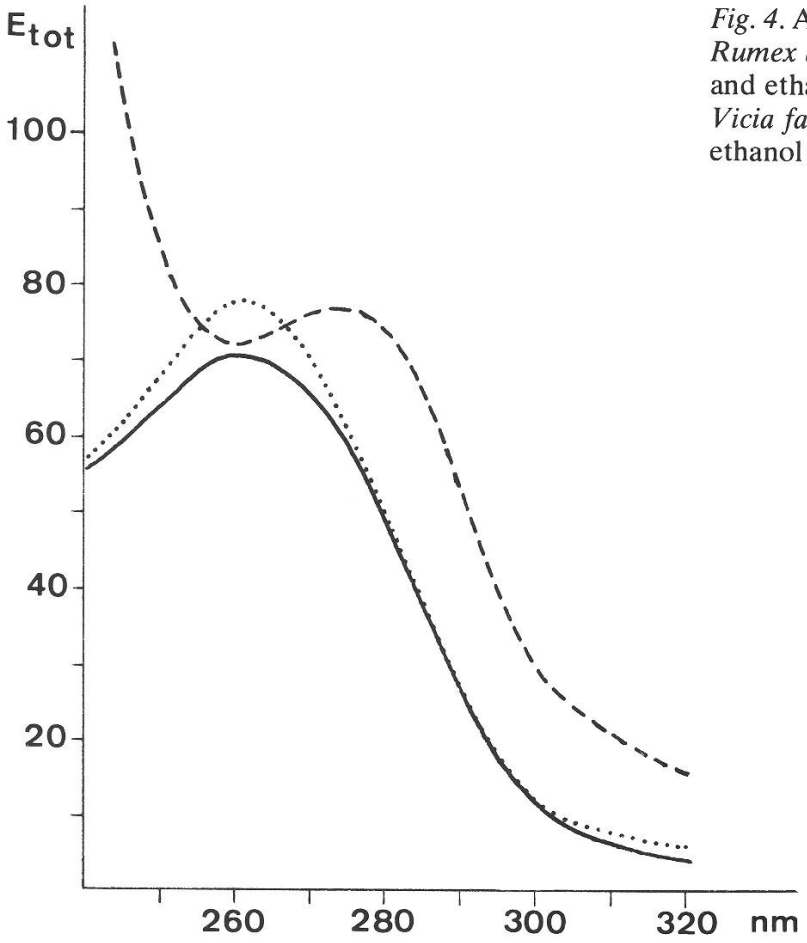

Fig. 4. Absorption spectra of cell nuclei of *Rumex acetosa* fixed in formalin (———) and ethanol-acetic acid (– – – –), and of *Vicia faba* (......, E_{tot} x 0.3), fixed in ethanol acetic acid.

Cytophotometry

Determination of the DNA content: the UV absorption of nuclei freed of cytoplasm by the squashing procedure was measured. In formalin fixed preparations a normal DNA absorption spectrum and the expected 2C–4C distribution was obtained (Fig. 4; table 1). EA fixed preparations yielded similiar values; the deviations, however, were much larger and the absorption maximum was at 275 instead of 260 nm. The result of absorption measurements on Feulgen-stained, formalin fixed cells coincides with that of the UV absorption; the 2C DNA content can thus be taken as 7 pg. Acetic acid fixation yields a much weaker Feulgen reaction; variation of the hydrolysis time did not improve this finding.

Determination of base content: Measurements of CMA and DAPI fluorescence made on ground leaf tissue *fixed in formalin* were reproducible, and a base content calculation based upon them is given in table 1. With both linear and exponential calculations (Langlois et al., 1980; Leemann and Ruch, 1982) the difference between male and female plants is more or less as expected and the values of the base contents lie within a reasonable range.

Table 1. DNA and base content of *Rumex acetosa.*

		Reference: *Vicia faba* (Leemann and Ruch, 1982)	*Rumex acetosa*		
			female: «euchromatin»	male: «eu + hetero-chromatin»	Y1 +Y2 «hetero-chromatin»
DNA	Chromosome length:				
	Autosomes		72%	72%	
	X		22%	11%	
	Y1			9%	17%
	Y2			8%	
	total		94%	100%	
	UV absorption (^{E}tot 260 nm)	26.0 pg		7.0 ± 0.6 pg	
	Feulgen (^{E}tot 550nm)	26.0 pg		7.0 ± 0.3 pg	
AT	DAPI	16.4 pg = 63%	2.24 pg = 61%[1] 62%[2]	2.6 pg = 65%[1] 64%[2]	82%[1] 76%[2]
GC	CMA	9.6 pg = 37%	1.43 pg = 39%[1] 38%[2]	1.4 pg = 35%[1] 36%[2]	18%[1] 24%[2]
DNA:	DAPI + CMA	26.0 pg	3.7 pg (92%[1] of male)	4.0 pg	

The calculation of the base content was carried out according to the formula AT + GC = $DAPI^{1/x}$ + $CMA^{1/x}$, for [1] x = 1 and [2] x = 2.

Though adequate for observation, the fluorescence of tissue *fixed in EA* did not yield satisfactory results. The deviations within and between slides were rather large, and while root tips from seedlings (as used for Fig. 2) showed strong fluorescence, tissue from grown plants fluoresced, especially with DAPI, much less strongly then expected.

In view of the UV absorption and Feulgen results the failure with tissue fixed in EA and the better results after formalin fixation is not too astonishing. *Rumex* probably produces a UV absorbing substance (Fig.4) which interferes with the DNA staining; by the aqueous formalin fixation enough of this substance may be removed to allow the DNA determination. The sum of CMA and DAPI being lower than the DNA value may indicate that some of the substance is preserved even with formalin fixation and weakens the fluorescence, or else, that the influence of formalin upon staining is not exactly the same in different species.

Since for many staining reactions, including DAPI and CMA, alcoholic fixatives are usually recommended, and since good chromosome spreads cannot be obtained with formalin, the use of *Rumex acetosa* as a model object for eu- and heterochromatin is somewhat limited. However, at least an approximative base content determination was possible with material fixed in formalin, and morphologically the double staining with the fluorochromes DAPI and CMA is very helpful for the localisation and identification of the sex chromosomes and the sex chromatin in interphases.

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Zusammenfassung

Mit Hilfe der Fluoreszenz-Doppelfärbung Chromomycin-DAPI lassen sich die heterochromatischen Y-Chromosomen von *Rumex acetosa* in Mitosen wie Interphasen (als Sexchromatin) leicht identifizieren. In den vorwiegend euchromatischen Zellkernen weiblicher Pflanzen beträgt der AT-Gehalt ungefähr 60%, in den Y-Chromosomen 80%. Ein 2C-DNA-Gehalt von 7 pg konnte bestimmt werden. *Rumex acetosa* produziert offenbar eine im UV absorbierende Substanz, die besonders nach Alkohol-Eisessig-Fixierung die DNA-Färbungen in einem gewissen Grade zu beeinträchtigen vermag.

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Dr. Ursula Leemann and Prof. Dr. Fritz Ruch
Department of General Botany
Swiss Federal Institute of Technology
Universitätstrasse 2
CH-8092 Zürich
Switzerland