Ribosomal gene numbers in Microseridinae (Compositae: Cichorieae)

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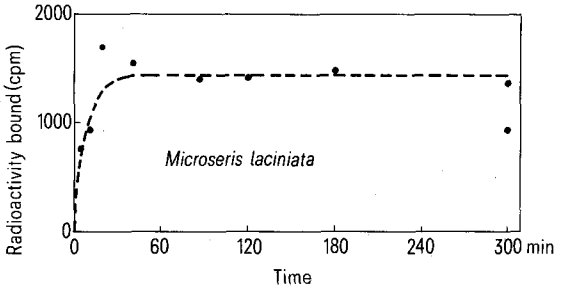
Summary. 8 species of the subtribe Microseridinae contain between 1100 and 3400 genes for 25 and 18 S ribosomal RNA. The gene numbers seem to evolve by discrete steps. Their trend follows a general reduction in genome size during the evolution of the annual species of *Microseris*, but numbers remain high in one of them and in *Agoseris grandiflora*. 2 species of *Pyrrhopappus* differ by a duplication of the ribosomal gene numbers; 5 S ribosomal RNA genes in 4 species are repeated roughly 10,000 times.

Plants of the subtribe Microseridinae constitute an ideal model system for the investigation of genome size evolution. Diploid species of *Microseris* all contain 18 chromosomes per nucleus, but there is a 3fold decrease in the nuclear DNA amount from primitive perennial species to specialized annuals[2]. This decrease is accompanied by clearcut morphological changes which can be studied by formal genetic analysis[3,4]. A similar trend is found in the related genus *Agoseris*[2]. *Pyrrhopappus*, which also belongs to the Microseridinae, contains twice as much DNA as the primitive species of *Microseris* in only 12 chromosomes. These are large and allow an investigation of the chromosomal basis of genome size changes. Recently we have compared the renaturation kinetics of thermally denatured DNA of these species[5]. The results suggest that the changes in genome size occur in discrete large steps. Fast, intermediate, and slowly renaturing fractions change to a certain degree independently of one another. Since these fractions are defined by the experimental conditions of renaturation, we present here determinations of the numbers of ribosomal genes in the same species. Ribosomal genes make up only a very small fraction of the genome, and one which is not representative for the genome as a whole, but their absolute number can be determined. Thus, they allow an independent analysis of quantiative changes during genome evolution. The observation that the ribosomal genes behave in a manner analogous to the bulk repetitive fractions complements and supports our picture of the way in which DNA fractions change quantiatively during species formation.

Material and methods. DNA was isolated from fresh young leaves of laboratory or garden-grown plants as described earlier[5]. Total DNA samples were denatured and loaded onto nitrocellulose filters (10 µg/filter). Radioactive RNA fractions used for hybridization were obtained from sterile cultivated seedlings of *Matthiola incana* (Cruciferae) labelled in vivo with (^{3}H) uridine (500 µCi/ml) for 48 h. Isolation and purification of 25 S, 18 S and 5 S rRNA (specific radioactivity: 25,000-90,000 cpm/µg) was carried out as described by Grierson[6] and Grierson and Hemleben[7]. The T_m-values of the DNA/RNA hybrids and the amount of DNA present on the filters at the end of the reaction was determined according to Grierson and Hemleben[7] and Ingle et al.[8]. For hybridization, the general method of Birnstiel et al.[9] was followed. 5 S rRNA hybridization was in the presence of unlabelled high-molecular-weight RNA in excess.

Results and discussion. The percentage of the genome coding for ribosomal RNA and the number of coding sequences for ribosomal RNA were determined by saturation hybridization with DNA on filters. The figure shows a typical experiment with *Microseris laciniata* DNA on filters incubated for various lengths of time with tritium labelled 25 S and 18 S rRNA isolated from the crucifer *Matthiola incana*. Saturation is reached after about 2 h. Therefore all saturation hybridization experiments with DNA from different species were carried out for 3 h in 2×SSC at 65 °C.



Saturation hybridization of *Microseris laciniata* DNA with ^{3}H- 25 S and 18 S rRNA (specific activity: 91 000 cpm/µg). Denatured DNA fixed to nitrocellulose filters was incubated with the radioactive RNA (2 µg/ml of each rRNA) at 65 °C in 2×SSC for 5-300 min. Filters were then washed, RNase treated and counted. The amount of DNA present on the filters was determined at the end of the reaction (5 µg/filter).

Comparison of the haploid genome size, percentage of double-stranded DNA coding for 5 S, and 18 S plus 25 S rRNA, gene numbers, and the percentage of intermediate DNA of 8 species of Microseridinae

Species	pg DNA (haploid)	5 S DNA (%)	Number of 5 S genes (haploid)	25 S+ 18 S rDNA (%)	Number of 25 S + 18 S genes (haploid)	Intermediate DNA %	Intermediate DNA pg	rDNA/ intermediate DNA (%)
Microseris laciniata	3.35	0.054	13,700	0.62	3,200	34	1.14	1.8
M. lindleyi	2.0	0.066	10,000	0.50	1,560	35	0.70	1.4
M. bigelovii	1.5	–	–	0.84	1,970	40.5	0.61	2.0
M. douglasii	1.4	0.084	9,000	1.34	3,000	24.5	0.34	5.5
M. elegans	1.4	–	–	0.50	1,100	–	–	–
Agoseris grandiflora	2.0	0.050	7,600	0.80	2,500	22	0.44	3.6
Pyrrhopappus multicaulis	5.5	–	–	0.40	3,400	40	2.20	1.0
P. carolinianus	6.2	–	–	0.20	1,900	39	2.41	0.5

The percentage of 18 S plus 25 S and 5 S rDNA was estimated from saturation hybridization data; the amount of the intermediate DNA fraction was determined from renaturation kinetics in 0.8 M Na^+ at 69 °C.

The saturation values were calculated from the double reciprocal plot of the data according to Bishop[10].

In the table the percentage of ribosomal DNA is shown for the plant species investigated. The number of genes coding for 25 S and 18 S rRNA was calculated for the haploid DNA content taking the molecular weight for 25 S rRNA as 1.25×10^6 daltons and for 18 rRNA as 0.64×10^6 daltons[7] or correspondingly the number of nucleotides as 5700. The amount of rDNA given in the table does not represent the actual total rDNA percentage, because it takes no account of the DNA transcribed in those parts of the prescursor rRNA molecule which are cut off during processing and of the non-transcribed spacer DNA. The restriction endonuclease pattern of soybean rDNA (Friedrich, Hemleben, Meagher and Key, in preparation) suggests that the amount would increase by about 37% provided that the non-transcribed spacer has the same size in Compositae.

The number of 5 S genes in the 4 species in which it was determined is about 10,000. There are roughly 3 times as many 5 S genes as there are ribosomal RNA cistrons for the large rRNAs. The 4 determinations allow no further conclusions, except that there is no proportionality between the number of 5 S genes and that of the ribosomal cistrons.

Numbers for the latter have been determined in 8 related species. For 6 of these, the exact nature of their relationship is fairly well established[5]. *Microseris laciniata* can be regarded as ancestral to the 4 annual species of *Microseris* and possibly to *Agoseris*. The main trend among these species is a reduction of genome size. In all of these except *M. douglasii*, the number of ribosomal cistrons is roughly proprotional to the total genome size and constitutes about 0.65% of the total genome. In *M. douglasii* the proportion is roughly double this amount. The simplest explanation for this is based on gene numbers. While 3 of the annual *Microseris* species have lost about half of their ribosomal genes, *M. douglasii* has retained (or regained) the ancestral number of 3000. The repetition frequency of the ribosomal genes make them part of the intermediate renaturing fraction as defined in the earlier paper[5]. Their behavior, however, is exactly the opposite of this fraction. While *M. douglasii* is the only annual that has reduced the amount of intermediate renaturing DNA relative to the slowly renaturing fraction by one-half, it is also the only one that has retained all of the ribosomal cistrons. Strikingly enough, *Agoseris grandiflora*, derived from *M. laciniata* by an independent route of evolution and still a perennial with a genome larger than that of *M. douglasii*, seems to have undergone about the same proportional changes among its genome fractions as *M. douglasii*. The genus *Pyrrhopappus* is only distantly related to the other species studied here. In terms of absolute numbers of ribosomal genes, it is similar to them. The 2 species studied appear to differ by a duplication of the ribosomal gene numbers. *P. carolinianus* with clearly more nuclear DNA than *P. multicaulis*, has the smaller number of ribosomal cistrons.

The results reinforce our earlier conclusion that genome evolution in the Microseridinae progresses by doublings and halvings of bulk fractions relative to one another. They add the observation that the ribosomal cistrons behave as an independent fraction. Particularly with respect to the bulk of the intermediate renaturing DNA, they can change their proportion, and they seem to do it by doublings and halvings. There is no obvious pattern concerning the number of ribosomal genes in all angiosperms[11]. The detailed investigation of a group of closely related species undertaken here shows that within such a group the evolution of ribosomal gene numbers follows a pattern that complements and supports our conclusions drawn from renaturation kinetics of bulk DNA fractions.

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In vitro studies on lysosomes radiosensitivity in different gaseous atmospheres

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Summary. Radiosensitivity of lysosomes was investigated in vitro in different gaseous atmospheres. Results show a higher sensitivity when X-irradiation was performed in nitrous oxide. Possible reasons for this observation are considered.

It is well known that lysosomes are very radioresistant in vivo[2]; however, biochemical tissue changes following X-irradiation make the fine analysis of experimental results difficult. Therefore many authors have investigated radiation effects in vitro on lysosomal suspensions, usually prepared from rat liver or rabbit neutrophyle granulocytes[3,4]. Aim of this research is the evaluation of the behaviour of mouse liver lysosomes after irradiation in different gaseous atmospheres (air, nitrogen, oxygen, nitrous oxide).

Materials and methods. Balb/c inbred mice, 3 months old, fasting for 24 h, were killed by bleeding. Homogenized livers were diluted with sucrose (8.5%; pH 7.2), 4 ml/g liver. Sediment from a first centrifugation at 2500 rpm for 25 min was discarded and supernatant restored to the starting volume and stored at 4 °C. Protein concentration was measured by the method of Lowry et al.[5]. Lysosomal suspension was subdivided in 2 parts: the 1st was used for the study of acid phosphatase release; the 2nd was lysed by Triton X 100 at a final concentration of 0.2% in order to