DNA distribution in chromosomes of *Lathyrus* species

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Abstract

Large changes in chromosome size and nuclear DNA amounts have occurred in the evolution of species within the genus *Lathyrus*, which are also reflected in the amounts of heterochromatin and euchromatin and of repetitive and non-repetitive sequences. The distributions of nuclear DNA in the chromosome complements of 4 *Lathyrus* species are compared. Although there is a doubling of the total amounts of nuclear DNA between highest and lowest of the four species the distribution among the chromosomes of each complement is approximately the same. Hence underlying these evolutionary changes there appear to be constraints upon changes in nuclear organization during speciation.

Introduction

Most species within the genus *Lathyrus* are diploids with the same chromosome number \(2n = 14\) although there are effective reproductive barriers between many species which prevent interspecific hybridization (Senn, 1938). Despite the large interspecific divergence which is accompanied by a fourfold increase in nuclear DNA amounts (Rees & Hazarika, 1969), the species show marked similarities in their chromosome shapes and karyotype arrangements within complements. The amounts of nuclear DNA within this genus are discontinuously distributed, species clustering into DNA groups separated by DNA intervals of approximately 3.95 pg (Narayan, 1982). The differences in nuclear DNA are due to quantitative changes in repetitive and non-repetitive components which are in the same ratio of approximately 4.1 to 1 through the evolution of the genus (Narayan & Rees, 1976; Rees & Narayan, 1977).

While the reproductive barriers between species and changes in chromosome size and in nuclear DNA amounts signify large interspecific divergence within this genus, the remarkable constancy in chromosome number, chromosome shape, karyotype arrangements, and in the repetitive and non-repetitive DNA ratio, suggests constraints upon changes in nuclear organization during evolution. In this paper the distribution of nuclear DNA in the chromosome complements of four *Lathyrus* species are compared and it is shown that although there is a doubling of total amounts of nuclear DNA the distribution of DNA among the chromosomes of each complement is about the same.

Material and methods

The four species investigated are *Lathyrus angulatus*, *L. articulatus*, *L. hirsutus*, and *L. tingitanus*. These were chosen at random and they covered the maximum DNA variation among the available species. According to the classification by Senn (1938) *L. articulatus*, *L. hirsutus* and *L. tingitanus* belong to three different subgeneric sections of *Lathyrus*. *L. angulatus* was not included in Senn's classification.
Measurement of total nuclear DNA in Lathyrus species

The amounts of total nuclear DNA were measured using Feulgen photometry. The method was first suggested by McLeish and Sunderland (1961). A modified method which minimized experimental error (Teoh & Rees, 1976) was used in this experiment. All DNA measurements were made on a Vicker's M85 microdensitometer. The four species were measured together with Allium cepa as control. A. cepa (33.5 pg) was used as a standard to convert the DNA estimates to absolute amounts. All DNA measurement experiments included two replications. Seeds received from different geographical regions formed the replicates for each species. At least three plants were measured in each replication and twenty 2C nuclei were scored in each root tip. Analysis of variance showed no significant difference between nuclei within root tips and between plants in replications.

Measurement of chromosome volume

The volumes of individual chromosomes were measured using the method suggested by Jones (1967). Excised root tips from germinating seeds were treated with 0.1% colchicine for three hours and fixed for 24 hours in 1:3 acetic alcohol. The root tips were stained in Feulgen and squashed out in acetocarmine. The lengths of the chromosomes and the widths of the chromatids were measured at C-mitosis just prior to the separation of chromatids. Measurements were made under an oil immersion lens using the Vicker's instrument eye-piece attachment with a moving scale which gave a high degree of accuracy. The chromosomes in each cell were measured individually for length and five chromatids in each cell taken at random to obtain mean chromatid width. Volume was then calculated from the total chromatid length (2X chromosome length) and average chromatid width assuming the chromatids to be cylindrical in form. The mean chromosome volumes were based on at least five cells from different plants.

Metaphase chromosome preparations were stained for C-bands using the method suggested by Vosa (1974). Most species in Lathyrus have constitutive heterochromatin at or near their centromeres. The volumes of constitutive heterochromatin and euchromatin were measured separately for each chromosome.

Measurement of DNA in chromosomes

Fixed root tips were stained quantitatively with Feulgen. Well-spread metaphase squashes were made in 45% acetic acid. The DNA contained in the 14 metaphase chromosomes was measured in an M86 microdensitometer. A special masking device was used to measure individual chromosomes. The mean DNA values are based on the measurements from at least two full metaphase plates. In the analysis of variance there were no statistically significant differences between homologous chromosomes within a chromosome complement. There were also no statistically significant differences between the DNA estimates obtained from different metaphase plates within each species. The DNA amounts obtained in arbitrary units were converted to picograms using the total DNA value estimated for each species from the interphase nuclei.

The total chromosome volumes of Lathyrus species are directly correlated with their nuclear DNA amounts. A comparison of the total chromosome volumes and the nuclear DNA amounts of 21 Lathyrus species showed highly significant regression (P < 0.001, Narayan (1982)). Using the volumes of individual chromosomes it was possible therefore to make an independent estimate of the total DNA contained in each chromosome. This estimate of chromosomal DNA in the present experiment again confirmed the DNA measurements obtained from Feulgen photometry.

Measurement of DNA in euchromatin and heterochromatin

Methods are not available to measure directly the amounts of DNA contained in the heterochromatin and euchromatin sectors of chromosomes. However, the DNA content of the two fractions can be estimated if the volume and density of each fraction are established. The interphase nuclei of Lathyrus species show distinct chromocenters. The number of chromocenters in most species is equal to the number of C-bands in the metaphase chromosomes. Using spot microdensitometry the densities of heterochromatin sectors were measured (Narayan & Rees, 1976). Densities of five euchromatic