Comparison of Cold-sensitive and C-banded Segments of Chromosomes in *Paris tetraphylla* A. Gray

A. Uchino and L. Wang

Department of Biological Science, Faculty of Science, Kumamoto University, and
Division of Natural Environmental Science, Department of Environmental Science, Graduate School of Science and Technology, Kumamoto University, Kumamoto 860, Japan

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The development of preferential staining methods has greatly facilitated studies on the linear differentiation of chromosomes. The cold induced effects on heterochromatin was discovered by Darlington and La Cour (1938) in *Paris polyphylla* and has been extensively investigated in plants of *Trillium* (Darlington and La Cour 1940, Haga and Kurabayashi 1952, 1954, Haga et al. 1974, etc.), *Scilla sibirica* (Baumann 1971), *Fritillaria* (Darlington and La Cour 1941, La Cour 1951), *Adoxa moschatella* (Geitler 1940), *Tulbagia* (Dyer 1963, Vosa 1966), and *Cestrum* (Dyer 1963). In these plants, cold treatment induces thinning and understaining in certain specific chromosome segments (cold-sensitive segments). The other segments remain normal in the degree of contraction and stainability. The mechanism underlying cold-induced H-segment formation is presently unknown. Several workers have applied the preferential staining methods (banding methods such as Q-, C-, G-banding, and so on) to heterochromatin of plant chromosomes and they found, in some cases, a correlation between the cold-sensitive segments and the positive banded segments. In some plants such as *Trillium erectum* (Caspersson et al. 1968), *T. grandiflorum* (Schweizer 1973), *Scilla sibirica* (Schweizer 1973, Vosa 1973), *Fritillaria lanceolata* and *F. recurva* (Schweizer 1973), and *Adoxa moschatella* (Greilhuber 1979), the positive banded chromosome segments were identical with the cold-sensitive segments. In *Paris polyphylla* (Filion and Vosa 1980) and some species of *Tulbaghia* (Vosa 1970), the heterochromatic segments induced by cold treatment appear as reduced fluorescence Quinacrine staining. On the other hand, Takehisa and Utsumi (1973) showed that the Giemsa staining method stained differentially not only the cold-sensitive segments but other regions of chromosomes in *Trillium kamtschaticum* as well. This was also found in plants such as two American species of *Trillium* (Chinnappa and Morton 1978) and five species of *Cestrum* (Berg and Greilhuber 1992, 1993a, b). Thus, a correlation between the cold-sensitive segments and positive banded segments is complicate and still obscure. The present paper describes a comparative study of the cold-sensitive segments and C-banded segments of chromosomes in *Paris tetraphylla*.

Materials and methods

*Paris tetraphylla* A. Gray (*Liliaceae*) were collected from the Miike population, Gokanosho, Kumamoto Prefecture, Kyushu. Root-tips were used for the analysis of the ordinary karyotype and young ovular tissues for the analyses of cold-sensitive and C-banded segments. The preparative procedures of respective analyses are as follows.

1. Analysis of the ordinary karyotype

The root-tips were pretreated with a 0.002 mol aqueous solution of 8-hydroxyquinoline for
3 hr before fixation with Carnoy's solution for 24 hr, macerated in 45% acetic acid-1 N HCl (1:1) solution at 60°C for 30 sec. and then squashed in 2% aceto-orcein solution.

2. Analysis of cold-sensitive segments

The flowers in thin plastic bags were chilled at 0°C for 96 hr. After cold treatment the ovules were dissected out, fixed in La Cour 2BE for 20 min, macerated in 1 N HCl at 60°C for 20 min, and squashed following the Feulgen procedure. Finally, preparations were made permanent by using a modification of the Conger and Fairchild dry-ice method (Uchino 1980).

3. Analysis of C-bands

The ovules which were dissected from flowers were treated with the same procedure as the ordinary preparation and squashed in 45% acetic acid solution. Coverslips were then removed by the dry-ice method and the slides were air dried for 2 to 3 days at room temperature. They were then immersed in a 4% Ba(OH)₂ solution at 35°C for 5 min, washed in distilled water for 10 min and incubated in 2 × SSC solution at 60°C for 2 hr. The slides were rinsed in distilled water and stained with 1% Giemsa solution (PBS, pH 6.8). Finally the preparations were rinsed in distilled water, air dried and mounted.

Results

1. Ordinary karyotype

*Paris tetraphylla* has a diploid chromosome number (2n) of 10. The five chromosomes constituting a genomic set were designated according to length as A, B, C, D, and E, A being the longest and E the shortest (Fig. 1a, Haga 1934). Among them, chromosome A was metacentric and B, C, and E were submetacentric, but D was subtelocentric. Chromosome D usually has a small satellite attached to the distal end of the short arm, but the appearance of the satellite and length of stalk were variable. Therefore, two kinds of karyotypes were distinguished among the plants examined: a karyotype with a homozygous pair of satellited chromosomes D and one with a heterozygous of satellited and non-satellited chromosomes. No karyotypes with accessory chromosomes were found in the present observations.

2. Cold-treated karyotype

All seven plants revealed the same patterns of cold-sensitive segments regardless of the difference of the ordinary karyotype (Fig. 1b). Namely, the cold-sensitive segment was confined to the distal half of the short arm of chromosome C and was not found anywhere in other chromosomes. Even the satellite region on chromosome D was not sensitive to differential reaction.

3. C-banded karyotype

Positive segments (C-bands) for preferential Giemsa staining were found in some regions of all chromosomes A to E (Fig. 1c). According to the location along the chromosomes, five classes of C-bands were distinguished: satellite, telomeric, centromeric, pericentric, and intercalary bands. If the size of bands are regarded as criteria, three kinds of bands were recognized: thick, thin, and granular bands. The karyotypes by the patterns of C-bands varied widely from plant to plant. The karyotype of the individual shown in Fig. 1c, for example, is explainable as follows. Chromosome A reveals one pericentric granular, one intercalary granular, and three telomeric bands in one arm and a thick band near the centromeric region in the other arm. A pair of chromosomes B is heterozygous owing to the difference of numbers and size of the bands. In chromosome C, pericentric bands were situated on each side of the centromere in
addition this centromeric band, next to a thick band in the distal region of the short arm which was located the cold-sensitive segment. The homozygous chromosome D has thick bands in the satellite and stalk, the whole short arm, and the nearby centromere in the long arm. Finally, chromosome E is a homozygous pair which has a pericentric band in the long arm.

Discussion

The ordinary karyotype observed in this study is congruous with previous reports (Gotoh 1933, Haga 1934, Kurabayashi 1952, Suzuki and Yoshimura 1986, Miyamoto and Kurita 1990), although the appearance of a satellite on chromosome D was variable. Furthermore, the appearance of cold-sensitive segment was identical with those in previous papers (Darlington and La Cour 1938, Kurabayashi 1952, Kurabayashi and Samejima 1953, Noda 1963). Namely, the cold-sensitive segment was confined to the distal half of the short arm of chromosome C and was not variable among plants (Fig. 1b). On the other hand, the C-banded karyotypes were variable from plant to plant. This was true in previous reports (Miyamoto and Kurita 1990, Miyamoto et al. 1991). C-bands were not found only in the cold-sensitive segment, but also in certain regions on all of chromosomes A to E (Fig. 1c). Thus, it was elucidated that the chromosomes of *P. tetraphylla* have reactive segments to both cold treatment and C-banding staining, or only to C-banding staining, in addition to normal euchromatic regions.

Several workers have elucidated that constitutive heterochromatin includes a multitude of types characterized by their sensitivity to cold treatment and by their differential reaction to preferential staining methods (Vosa 1970, 1973, 1976, Fiskesjo 1974, Greilhuber 1975, Yen and Filton 1977, Greilhuber and Speta 1978, La Cour 1978, Sato et al. 1979, Deumling and Greilhuber 1982, etc.). Vosa (1970) classified heterochromatin into four main types, showing
all possible combinations of positive and negative cold-induced effect “starvation” (St. + or St. −) and enhanced or reduced fluorescence (Fl. + or Fl. −). However, the relationship between the four different classes of heterochromatin was not clear. Recently, on the basis of the characteristic response of heterochromatin to differential staining with CMA, DAPI, D 287/170 and C-banding, N-banding, silver impregnation and others, Berg and Greilhuber (1992, 1993a, b) discriminated constitutive heterochromatin into four main classes in five species of Cestrum; (1) CSRs (cold-sensitive regions), (2) NORs and associated heterochromatin, (3) non-nucleolar CAM-positively fluorescing bands and (4) indifferently fluorescing, positively C-stained bands. Only their CSRs react to cold treatment although all classes of heterochromatin were positively C-staining. Furthermore, banding behaviour of CSRs indicated AT-rich and of NORs GC-rich constitutive heterochromatin. These strongly support the present findings, and the cold-sensitive region of P. tetraphylla chromosomes might be AT-rich constitutive heterochromatin.

**Summary**

A comparative study of the cold-sensitive segment and C-banded segments of chromosomes was made in Paris tetraphylla (2n=2x= 10). The cold-sensitive segment was confined to the distal half of the short arm of chromosome C out of five chromosomes A to E. On the other hand, C-bands were not only found in the cold-sensitive segment but also regions of all chromosomes A to E. Thus, it was elucidated that the heterochromatic regions of this species consist of reactive segments to both cold treatment and C-banding staining and only to C-banding staining. The cold-sensitive segments of Paris tetraphylla chromosomes might be AT-rich constitutive heterochromatin.

**References**


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