Chromosomal Changes Observed in Natural Populations of *Paris tetraphylla* A. Gray

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Paris tetraphylla A. Gray is a perennial plant which is usually found under light forests in mountainous regions of Hokkaido. It propagates sexually by seeds and vegetatively by extension and branching of rhizomes. In natural conditions this plant usually grows in groups. The size of the groups varies with conditions of their habitat. They usually consist of 5~6 to 50~60 terrestrial stems. The individual group cannot be distinguished by means of external appearance whether it is a homogeneous one derived from one and the same seed or a heterogeneous one grown from several different seeds.

In May 1951, plants of this species were collected from 6 localities near Sapporo. The flower bearing terrestrial stems belonging to several neighbouring groups in each locality were collected at random. The structural changes of chromosomes were observed by means of the differential reaction of chromosome segments.

Material and method

Terrestrial stems whose flowers are just prior to opening were chosen for this investigation. They were kept at 0°C for 48 hours. After this cold temperature treatment the ovaries of these flowers were fixed with La Cour 2BE. The chromosomes at metaphase were observed in the mitosis of these ovular tissues. The cytological preparations were made after the same method as that described previously (Kurabayashi '48).

Observation

Paris tetraphylla have five pairs of somatic chromosomes designated A, B, C, D and E respectively according to their length (Haga '34). The distal half of the short arm of chromosome C of this plant reveals the differential reaction, viz. under charging of nucleic acid in chilled condition. In the plants obtained from Maruyama (Maruyama-B: 1~4 in table 1), this differential region consists of four componental segments (Kurabayashi '48). The chromosomes of all the plants revealed the differential reaction confined only to the distal part of the short arm of chromosome C (see Figs). Six types (I~VI in Fig. 16) were distinguished morphologically

by the patterns of the differential reaction. Judging from the configurations of these differential patterns found in various groups, it may be seen that the structural variations in this region might be derived through deficiencies of one to three out of the above mentioned four componental segments of the differential region (see Figs. 1~16).

Chromosome D of this plant has a small satellite attached to the distal end of the short arm. This satellite often shows various appearance, but this variation bears no relationship either with the temperature condition or with the structural changes in chromosomes, because it occurs in the dividing figures of one

Figs. 1~9. Chromosome C (left side) and D (right side) at metaphase in the chilled ovular tissues of *Paris tetraphylla* obtained from Maruyama-A (1~4), Maruyama-B (5), Maruyama-C (6~8), Takinosawa (9), (to be continued to Figs. 10-13).

and the same individual (Kurabayashi '48 and his Figs. 5~9). Among the plants examined in the present investigations, however, some individuals were found to have chromosome D with none or, an extremely small satellite which scarcely changes its morphology in different temperature conditions. Chromosome D with larger-sized satellite is designated

Figs. 10~13. Nopporo (10, 11) and Otaru (12, 13), respectively. The Roman numerals written under chromosomes C and the signs under chromosomes D represent the types of the corresponding chromosomes, respectively. $\times 1700$.

here with D^+ and the one with none of extremely small satellite with D^- respectively (Figs. 1~13, 15).

None of chromosomes A, B and E revealed structural variation detectable with the method employed in the present study. Accordingly the chromosomal constitutions in natural

populations of this plant were investigated with chromosomes C and D. The results were summarized in the following table 1 with regard to the types of chromosomes C and D.

It is seen clearly in this table that in each group, the individuals belonging to one and the same group have the identical chromosome constitution,

Figs. 14~15. Chromosomes in the chilled ovular tissue of *Paris tetraphylla* obtained from Maruyama-B: 1 (C-III C-III, D^+ D^+) (upper) and Maruyama-A: 4 (C-II C-VI, D^+ D^-) (lower), respectively. $\times 1600$.

Figs. 16, 17. Schematic representation of the short arms of chromosome C (Fig. 16) and D (Fig. 17) distinguishing the six types of chromosome C and two of D, respectively.

Table 1. Chromosome constitution in *Paris tetraphylla* A. Gray

Locality	Clone No.	Chromosome type				Number of plants analysed
		C	C	D	D	
Maruyama-A	1	I	II	+	-	4
	2	II	III	+	-	5
	3	II	II	+	+	5
	4	II	VI	+	-	5
Maruyama-B	1	III	III	+	+	23
	2	III	III	+	+	29
	3	III	III	+	+	10
	4	III	III	+	+	6
	5	II	III	+	+	5
Maruyama-C	1	III	VI	+	-	4
	2	II	VI	+	+	4
	3	II	II	+	-	5
Takinosawa	1	II	III	+	+	5
Nopporo	1	V	V	+	+	5
	2	V	V	+	-	5
Otaru	1	II	III	+	+	4
	2	IV	V	+	+	4

while there are sometimes differences among different groups. Among the groups listed in the table 1, nine were heterozygous for chromosome C, and six were heterozygous for chromosome D.

Discussion

As indicated in the table 1, the chromosomal constitution differs according to localities as well as to groups in one and the same locality, except the four groups in Maruyama-B. Each group is heterozygous with respect to chromosome C or D, except Maruyama-A : 3, -B : 1, 2, 3 and 4, and Nopporo : 1, which are homozygous respectively. However, all the individuals that belong to one and the same group have the identical chromosomal constitution. Accordingly, it is reasonable to consider that each group consists of a single clone. This judgment is not always correct, because in the

population consisting of individuals with identical and homozygous chromosomal constitution, all clones in the population must have one and the same chromosomal constitution in so far as the population is isolated from others and no chromosomal mutation is allowed to happen or to persist and propagate. Such may be the situation of the groups in Maruyama-B: 1, 2, 3 and 4. In this locality the habitating area of this plant is restricted to a narrow belt along a small stream, and this area is isolated from neighbouring ones by geographical and ecological barriers (Fig. 18).

Fig. 18. Eco-geographical map of the distribution area of *Paris tetraphylla* at the foot of Mt. Maruyama. The small circles indicate the individual groups of this plant examined in each area (Maruyama-A: 1~4, B: 1~5 and C: 1~3). The areas encircled by broken lines are those having profitable conditions for the habitation of this plant. In the left side area, only five groups of this plant were found and the maximum number of the groups here may certainly be less than ten. The right side area is far wider than the former and contains numerous, perhaps some hundreds of, groups.

Based on the facts described above it becomes evident that the method of propagation in the natural population of *Paris tetraphylla* differs from that of *Trillium kamtschaticum* which belongs to a related genus. In *T. kamtschaticum*, natural populations consists of plants propagating by seeds (Haga and Kurabayashi, in press), while in *Paris tetraphylla* vegetative propagation by rhizomes takes place fairly conspicuously. How the difference of the method of reproduction among these plants influences the constitution of natural population of them has not yet been examined.

At present, it is impossible to explain the mechanism of the chromosomal mutation detected in the present material. Judging from the configuration of the differential regions, it seems that the chromosome C belonging to any one of the six types may be derived from one of the others by the deletions or the duplications of the componental segments of the differential region (Figs. 1~13, 16). It was demonstrated by Darlington

and La Cour ('40) that deletion and duplication are induced by the delayed and weak separation and the fusion of the differential regions locating at the distal end of chromosomes during mitotic anaphase in chilled condition. These chromosomal changes may also be brought about by the breakages and reunions of chromonemata during resting stage which result in the knot- and ring-formations (Husted '37 and Haga and Kurabayashi, in press). However, none of these phenomena has yet been detected in the present material. It was observed that daughter satellites rarely fuse at mitotic anaphase and are lost being broken at the sat-constrictions (Kurabayashi, unpubl.). Therefore, it seems that a chromosome D⁻ may be derived from a chromosome D⁺ by the loss of its satellite.

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Summary

Mitotic chromosomes of *Paris tetraphylla* ($2n=10$) in chilled condition were observed in metaphase of the plants belonging to seventeen groups gathered from six localities. The differential segments which manifest undercharging of nucleic acid in low temperature condition were found restrictedly at the distal region of the short arm of chromosome C. According to the patterns of this region, six different types were distinguished as for this chromosome. Chromosome D was the only satellited chromosome among the complements of the genom of this plant. As for this chromosome two different types were distinguished according to the size of this satellite.

The chromosome composition of the plants of each of the seventeen groups was analyzed with respect to the types of chromosomes C and D. The results obtained are as follows; 1) In ten groups out of seventeen, the plants had chromosome C or D in heterozygous condition, and in the remaining six they have them in homozygous condition. 2) The chromosomal constitution differs not only from locality to locality but also, with four exceptions, from group to group. 3) All the individuals belonging to one and the same group have the identical chromosomal constitution.

Based on these results it was assumed that in the natural population of *P. tetraphylla*, vegetative apomixis takes place far more conspicuously than in that of *Trillium kamschaticum*, which propagates almost exclusively by sexual reproduction.

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