Differential reactivity of the specific chromosome segments in *Paris**

By

MASATAKA KURABAYASHI

(With 10 text-figures and 2 tables)

In chilled condition, specific chromosome segments were found in mitotic chromosomes of *Paris vert'cillata* MB. and *P. tetraphylla* A. GRAY. These segments revealed the same structure as the differential segments found, under low temperature, in certain other plants (DARLINGTON and LA COUR '38, '40, '41, GEITLER '40).

In the present study, the structure of these segments and the influence of temperature upon their differential reactivity were investigated.

Material and method

Both of the plants were obtained directly from natural population at the foot of *Mt. Maruyama* near the city of *Sapporo*. Cytological preparations were made following FEULGEN'S technique modified by the present writer. The modification is as follows:

1. Carpels were removed and young ovules were fixed in LA COUR 2BE ..... 25 min.
2. Rinse in running water ..... 10 min.
3. Hydrolyse in 1 N HCl at 60°C ..... 25 min.
4. Rinse in running water ..... 5 min.
5. Stain with leuco-fuchsin ..... 45 min.
6. Rinse in running water ..... 5 min.
7. Smear and mount under pressure in 45 percent acetic acid.

Mitosis was observed in young ovular tissues. In the experiments on the influence of temperature the plant bodies cut from the rhizomes were transferred into thermostats adjusted at desired temperatures. After the desired duration of the temperature treatment cytological preparations were made following the procedure described above.

* Essentials of the present paper were formerly published in Japanese in 1948 and 1949.

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Experiments and observations

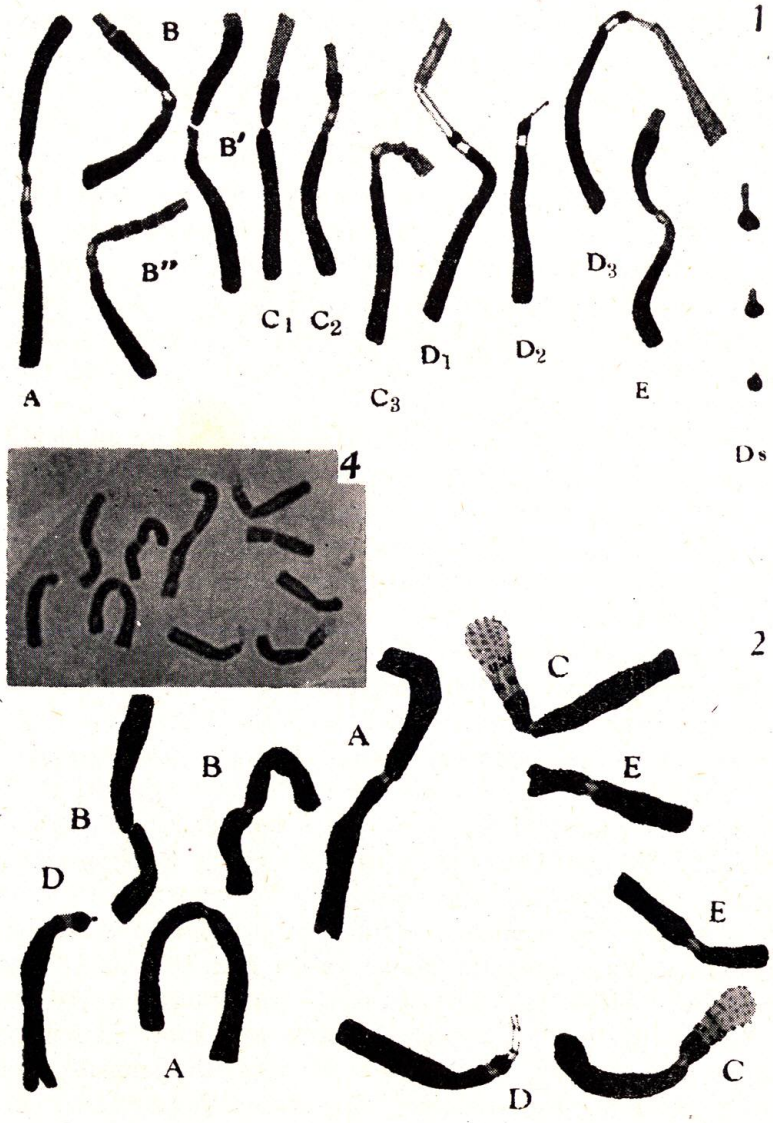
Among the population plants of *P. Verticillata* (2n=10) heterozygosis has been found for same pairs of homologous chromosomes (HAGA '37): viz., chromosomes C and C^- the short arm of the latter being shorter than the former: chromosomes D, D^- and D^+, the first having a satellite attached to the short arm, the second one and the third one which is longer than that of the first.

Four different karyotypes, of which three were diploids and one triploid, that is, CCDD, $CCDD^-$, CC^-DD^+ and CCCDDD (HAGA '37) were examined with regard to the differential reactivity.

After the plants were kept at 0°C for three days, differential segments revealed characteristic features in every chromosome. The position, size and the chromaticity of the segments are shown in Figure 1. Chromosomes A and E were homozygous having an identical pattern of the segments in all the plants examined. The differential reactivity of the short arm of chromosome B was somewhat unstable, revealing itself most often in B–state, next in B′–state and least in B″–state (Fig. 1). The chromaticity of the intercalarily located short differential segments varied not only from plant to plant and from cell to cell in the same preparation but also among the homologous chromosomes in the same nucleus. Accordingly this variation seems not to be caused by the structural difference but to be due to the smaller size and the greater affinity for the nucleic acids of the inserted specific segments as compared with the other differential segments.

As to chromosome C, three different type, C_1, C_2 and C_3, were distinguished in chilled condition. Under non-chilled condition C_1 and C_2 were classified into a single type, chromosome C, and C_3 into chromosome C^-. The entire length of the satellites of chromosome D consisted of a differential segment. Chromosome D^+ (D_3 in Fig. 1) had a longer understaining satellite than chromosome D (D_1 in Fig. 1) in chilled condition. A small differential segment was seen at the distal end of the short arm of chromosome D^- (D_2 in Fig. 1), which has no satellite in the non-chilled condition (HAGA '37, '42). The size of the small distal segment (Fig. 1, Ds) varied from cell to cell in the same preparation. Such instability of size was also seen at the distal end of several chromosomes in *Trillium kamtschaticum* kept at 0°C (HAGA and KURABAYASHI, unpublished).

The four karyotypes distinguished under non-chilled condition:



Explanation of Figs. 1, 2, 4. 1. Mitotic metaphase chromosomes of ovular tisste of *P. verticillata* kept at 0°C for three days. ×2600 2. Mitotic metaphase in a cell of ovular tissue of *P. tetraphylla* kept at 0°C for four days. 4. The same mitotic metaphase as figured in Figure 2. × 1000

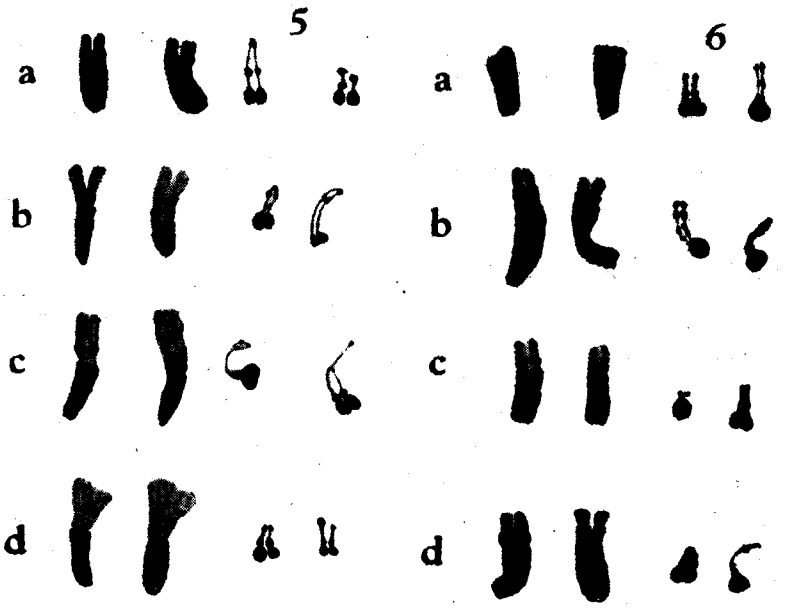
CCDD, CCDD$^-$, CC$^-$DD$^+$ and CCCDDD by HAGA's classification, will be redesignated by the patterns of the differential segments as follows: $C_1C_2D_1D_1$, $C_1C_2D_1D_2$, $C_1C_3D_1D_3$, and $C_1C_2C_3D_1D_1D_1$ respectively.

The chromosomes of *P. tetraphylla* (2n=10) showed no structural hybridity either in the non-chilled (HAGA '34) or in the chilled condition. The plants kept at 0°C for four days disclosed differentiated chromosomes. The specific segments were confined at the distal half of the short arm of chromosome C. No other segment showed differential reaction (Figs. 1, 2 and 4). Chromosome D of the present plant is furnished with a satellite at the distal end of its short arm. The satellite was seen variable in its morphological features (Figs. 3, 5–9). But the morphological instability of this appendage bears no relation to temperature.

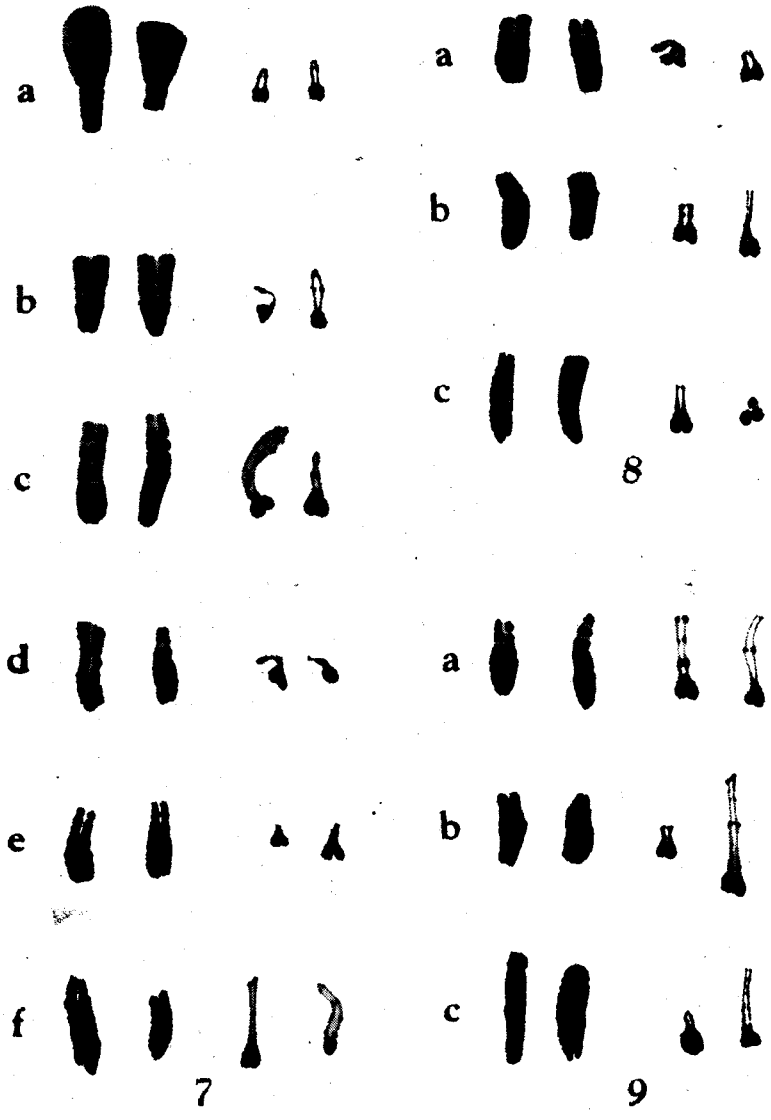
The mitotic chromosomes of this plant fixed under semichilled condition in the field, i.e. on a cold day in May* also revealed differential reaction but in less pronounced features than those revealed at 0°C (Fig. 3). The following experiments were then undertaken to examine the causal relationships between the differential reactivity to the environmental temperature.

Two series of experiments were carried out to make clear the effect of low temperature upon the differential reactivity. In the first series twenty four plants were transferred from field condition into a thermostat kept at 18°C. Seventeen hours after the transference, no differential segment was seen in any of the mitotic figures. A dozen of the plants were then transferred into a thermostat adjusted at 0°C and the remaining half into one adjusted at 10°C. Fixations were made at intervals of each one to two hours after the transference to ascertain the effect of low temperature. Differential reaction was manifested as early as one hour after the transference into 10°C as well as to 0°C temperature. Concomitant with the time elapsed characteristic features of the differential segment became more and more remarkable, the final state being reached, at 0°C, 10 hours after the transference. Further treatment under the same condition lasted as long as for four days showed no further effect upon the morphological features of the differential segment (Figs. 2, 4 and 5d). Transference into 10°C also revealed the differential segment, but with transitional features between the

* According to the record of the Meteorological Station of *Sapporo*, the atmospheric temperature during the precieding twenty four hours before fixation varied from 4.1 to 7.7°C with an average of 6.0°C.



Explanation of Figs. 3, 5–6. 3. Mitotic anaphase in a cell of stigma tissue of *P. tetraphylla* under field condition. Successive states in differential reaction in two cases of transference from high to low temperature. Short arm pairs of chromosome C (on the left) and D (on the right) from a mitotic metaphase of *P. tetraphylla* are shown alined. 5. The transference from a thermostat kept at 18°C for 17 hours into a thermostat adjusted at 0°C. Hours elapsed after the transference are 1, 3, 8 and 10 hours respectively in a, b, c and d. 6. Transference from the same pretreatment into a thermostat adjusted at 10°C. Hours elapsed after the transferense are 1, 5, 7 and 48 hours respectively in a, b, c and d. ×2600



Explanation of Figs. 7–9. Successive states in reversal of the differential reaction in the three cases of the transference from low to high temperature. 7. Transference from a thermostat kept at 0°C for 44 hours into a thermostat adjusted at 10°C. Hours elapsed after the transference are 0, 2, 4, 7, 9 and 48 hours respectively in a, b, c, d, e and f. 8. Transference from the same pretreatment at 0°C into a thermostat adjusted at 20°C. Hours elapsed after the transference are, 2, 5 and 48 hours respectively in a, b and c. 9. Transference from the same pretreatment at 0°C into a thermostat adjusted at 30°C. Hours elapsed after the transference are $^1/_2$, 2 and 5 hours respectively in a, b and c. × 2600

initial and the final state (Fig. 6).

For the sake of convenience in description, the writer classified the morphological features of differential reaction, arbitrarily, into seven stages, I–VII. The first represents the initial and the last the final state (Fig. 10). According to this seheme, all the results obtained in the first experiment are summarized in Teble 1. It is worthwhile to mention the fact that the differential reaction is much accelerated by low temperature, 0°C, the final state being reached as early as 9 to 10 hours after the transference. Whereas, at the higher temperature, 10°C, the reaction proceeds slowly, ceasing at a transitional state even 48 hours after the transference.

TABLE 1

Effect upon the differential reactivity of transference from high, 18°C., to low temperature

Transference into	Hours Elapsed after the Transference											
	1	2	3	4	5	6	7	8	9	10	24	48
0°C	III–IV	IV	V		V–VI		—	VI	VI–VII	VII	VII	—
10°C	I–II	—	II–III	III–IV		IV	IV–V	—	—	III–IV	III–IV	

TABLE 2

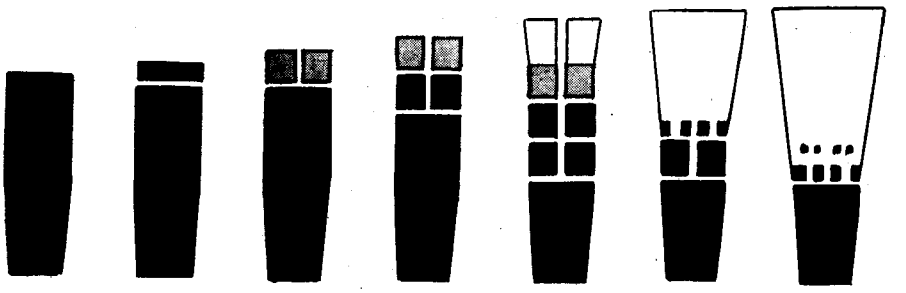
Effect of high temperature upon the reversal of the differential reaction

Transference into	Hours Elapsed after the Transference											
	1	2	3	4	5	6	7	8	9	10	24	48
10°C	VII	VI	VI–V	—	—	V–IV		—	—	IV–III		—
20°C	VII–VI	V	IV	IV–III	III	—	—	—	—	II–I		—
30°C	V–IV*	IV–III	III		III–II							

* Exceptional case: 1/2 hour after the transference.

Next, experiments were carried out in a reverse way. Some thirty plants were transferred from field condition into a thermostat adjusted at 0°C. They were kept in the thermostat for 44 hours. After this pretreatment differential segment was seen exclusively and only in the final state of the reaction. Then the plants were divided into three plots and transferred into three thermostats adjusted at 10°C, 20°C and 30°C respectively. Ovules werc fixed at intervals of each one to two hours to clarify the effect of transference. All the results are sum-

marized in Table 2, employing the same scheme as adopted in the preceeding table. It is found that there happens a reversal of the differential segment into the initial features. The reversal is complete, under higher temperature, 20°C, 24 hours after the transference. The rapidity in the reversion is marked under the higher temperature. At lower temperature, 10°C, the reversion proceeds gradually and is incomplete even after 48 hours (Figs. 7–9).



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Explanation of Figs. 10. A scheme showing successive morphological stages of differential reaction. The short arm of chromosome C of *P. tetraphylla*.
I: initial appearance. VII: final state of differential reaction.
II–VI: transitional states from the initial to the final state.

Structure of the differential segment

The differential segment of the short arm of chromosome C of *P. tetraphylla* is composed, at least, of four constituent segments, with the most easily differentiating portions intervening (cf. Figs. 5b, 7c, 8a and 9a). Hereinafter, each of the four segments will be referred to as the first, second, third and the fourth segment, respectively, from the most distal one. Every one of these segments shows its specific grade of staining capacity. The first is the weakest in chromaticity and the second the next. The third and the fourth are the strongest in affinity to stain, these two segments remaining as a number of deeply staining granules even in the final state of the differential reaction (Figs. 2, 4, 5d and 7a). In the first stage of the differential reaction, the first segment shows a reduced susceptibility to staining (Figs. 5a and 6a). This reduction in stainability progresses from the first segment to the

fourth one with progression in the differential reaction (Figs. 5, 6). In the final state, the first and the second segments are no longer distinguishable from the intervening unstainable portions (Figs. 2, 4, 5d 7a). Thus the differential segment changes its morphological features depending upon the stages of differential reaction, which proceeds progressively not in the fashion of all-or-nothing reaction. This is an important point not emphasized by the previous workers (DARLINGTON and LA COUR '40, '41). For this reason, a pair of differential segments in one and the same mitosis frequently shows discrepancies in the minor morphological features (Figs. 5–9). But such a difference between the partners implies nothing other than slight discrepancies in the stage of the reaction. This point is reinforced as one surveys the difference in mitosis in the same individual. The present conclusion raises a question as to the statement that structural hybridity is detectable due to the suspected difference in a pair of differential segments (DARLINGTON and LA COUR '40).

As pointed out by DARLINGTON and LA COUR ('40, '41), differential reactivity of specific chromosome segment reveals itself "only at marginal temperatures and seems to be conditioned by (a) lower supply of nucleic acid in the nucleus, and (b) a lower competitive strength or demand of the inert genes for nucelic acid in the presence of an insufficient supply" ('40 p. 210). With regard to spiralization, the differential segments "are the same length when undercharged as when normally charged". Therefore, "nucleic acid seems to have no necessary relation to spiralization" ('40 p. 209).

The latter conclusion, however, is disputable. For in the present case of *P. tetraphylla*, the differential segment increases its relative length, in the final stage of the reaction. Relative length in percentage of the short arm of chromosome C to the entire length is approximately 21–22 under the normal temperatures (HAGA '34). Whereas, in the chilled condition it is approximately 38 percent in Figure 3 and 38 and 44 percent in the lower and upper members respectively in Figures 2 and 4. Such a marked increase is obviously bue to the alteration related with the differential reaction. The diameter of the differential segment is reduced to a considerable degree in the primary stage as described by the previous workers (Figs. 3, 5–9). However, in the final stage the differential segment shows an unstained expanded feature, with scattering deeply stained granules in the third and the fourth component segments (Figs. 2, 4, 5d, 7a). In contradiction to the previ-

ous conclusions these two facts suggest unravelling or imperfectness in spiralization of the affected region. This feature of the final stage closely resembles the one end of chromosome C of the salivary gland cell of *Sciara ocellaris* (METZ '35).

Conclusion

As has been concluded by previous workers, differential reaction is characterised by the undercharging of nucleic acid at the specific chromosome segment. Differential segments revealed in *P. verticillata* and *P. tetraphylla* show their own inherent affinity to the nuclic acid, differing chemically from the non-differential segments. Thus the differential reactivity is conditioned by an internal property inherent to the specific chromosome segment, certainly by the genetical inertness. The constancy in the position, size and the features of the differential segment can be explained only on this genetical differentiation.

In is a well-established fact that the differential reactitivity is conditioned by environment, that is, by the low temperature near 0°C. In the present study with *P. tetraphylla* the differential reactivity is manifested even under a rather high temperature, 10°C, although in an imperfect manner. Differential reactivity dissappears when the plants are transferred from chilled condition to the initial environment. Rapidity in reversion from the differentiated to the initial state is accelerated by the higher temperature. On the contrary, differential reaction is completed more rapidly under lower temperature. All these facts suggest a series of biochemical reactions which are responsible for the morphological differentiation of the specific chromosome segment. Thus naturally the differential reaction is not all-or-nothing reaction as we have seen gradual transitional stages, in the morphological features, from the initial to the final one.

In conclusion, the writer wisher to express his cordial thanks to Prof. HAJIME MATSUURA and Prof. TSUTOMU HAGA for their valuable suggestions and criticisms in the course of the present study.

Summary

1. Differential segments of chromosomes are described for *Paris verticillata* MB. (2n=10) and *P. tetraphylla* A. GRAY (2n=10), which reveal their undercharging in nucleic acid at low temperature. All the chromo-

some complements of the former plant have differential segments. In the latter plant, the segment is confined to the distal half of the short arm of chromosome C, no other segment showing differential reactivity. Constancy in the position, size and features of these segments may be explained by the genetical differentiation, certainly by the inertness, of the specific segments. Thus differential reactivity is conditiond, on the one hand, genotypically.

2. Differential reactivity is seen only under low temperature conditions. It disappears shortly after the transference from chilled to temperate condition. The rapidity in reaction and in reversion is functional to temperature. Accordingly, the differential reactivity is, on the other hand, conditioned by environmental factors.

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